WEST Search History

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DATE: Tuesday, June 20, 2006

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR	
☐	L1	oxyrase	59
☐	L2	L1 near5 (supplement$ or modif$ or add$ or includ$ or more or alter$)	23
☐	L3	oxyrase$	59
☐	L4	preserv$ or inhibit$ or antagon$ or inactivat$	1557115
☐	L5	L4 near10 l2	1
☐	L6	L4 and l2 not l5	17
☐	L7	cyanide or sodiumcyanide or azide or sodiumazide or naazide ro na-azide or na-cyanide or nacyanide	1939379
☐	L8	oxydish$ or oxyrase$ or oxygen-scaveng$	646
☐	L9	sterile near membrane near fragments	22
☐	L10	oxygen near transfer near system	82
☐	L11	l8 or l9 or l10	731
☐	L12	L11 same l7	2
☐	L13	cyanide or sodiumcyanide or azide or sodiumazide or naazide or na-azide or na-cyanide or nacyanide	119517
☐	L14	L13 same l11	2
☐	L15	nan3 or na-n3 or (n near 3)	242880
☐	L16	L15 same l11	1

END OF SEARCH HISTORY

2. The bilirubin calibrator solution of claim 1 which further comprises catalase.

3. The bilirubin calibrator solution of claim 2 wherein the catalase is present in an amount up to about 150 units/ml.

4. A method for stabilizing an aqueous bilirubin calibrator solution comprising:

preparing a matrix containing:

between about 0.9 and 1.1 weight percent ethanol;

between about 5 and 6.4 weight percent polyoxyethylene 23 lauryl ether;

bistrispropane at about 0.1M;

between about 0.1 and 0.53 weight percent glucose;

between about 0.0016 and 0.024 weight percent butylated hydroxy toluene;

d-lactate hydrogen donor at between about 5 and 30 mM; and

between about 1 and 10 weight percent albumin;

adjusting the pH of the matrix to from about 7.7 to about 7.85;

adding into the matrix sterile membrane fragments derived from bacteria having membranes containing an oxygen transfer system which reduces oxygen to water so as to obtain between about 0.3 and 0.6 units/ml of activity and thereafter excluding air from contacting said matrix;

adding to the air excluded matrix catalase in an amount up to about 150 units/ml;

after oxygen is removed from said matrix by the oxygen transfer system, adding into said matrix sufficient glucose oxidase to obtain between about 10 and 27 units/ml;

adding into said matrix bilirubin dissolved in 0.1M NaOH so as to obtain between about 1 and 30 mg/dl of bilirubin in a bilirubin containing matrix, said dissolved bilirubin being substantially clear; and

sealing said bilirubin containing matrix in a reclosable, air tight container.

5. A stabilized aqueous bilirubin calibrator solution comprising:

between about 1.0 and 30 mg/dl bilirubin;

bistrispropane at about 0.1M;

between about 0.1 and 0.53 weight percent gluconic acid generated in situ from glucose;

between about 4 to about 6 weight percent albumin;

d-lactate hydrogen donor at between about 3 and 20 mM; and

between about 0.3 and 0.6 units/ml sterile membrane fragments derived from bacteria having membranes containing an oxygen transfer system which reduces oxygen to water;

wherein said stabilized bilirubin calibrator solution has a pH from about 7.7 to about 7.8 and has a storage stability of at least six months at from about 2.degree. to about 8.degree. C. and at least four days at about 45.degree. C., said calibrator solution further having an open vial stability of at least three weeks at from about 2.degree. to about 8.degree. C.

6. The bilirubin calibrator solution of claim 5 which further comprises up to about 0.024 weight percent butylated hydroxy toluene, from about 0.95 to about 1.0 weight percent ethanol, and from about 5 to about 6.4 weight percent polyoxyethylene 23 lauryl ether.

7. A stabilized aqueous bilirubin calibrator solution comprising:

between about 1.0 and 30 mg/dl bilirubin;

between about 0.1 and 0.53 weight percent gluconic acid generated in situ from glucose;

between about 1 and 10 percent albumin;

between 0.3 and 0.6 units/ml sterile membrane fragments derived from bacteria having membranes containing an oxygen transfer system which reduces oxygen to water; and

a substrate for said oxygen transfer system;

wherein said stabilized bilirubin calibrator solution has a pH from about 7.4 to about 8.1 and has a storage stability of at least six months at from about 2.degree. to about 8.degree. C. and at least four days at about 45.degree. C., said calibrator solution further having an open vial stability of at least three weeks at from about 2.degree. to about 8.degree. C.

8. The bilirubin calibrator solution of claim 7 which further comprises catalase.

9. The bilirubin calibrator solution of claim 8 wherein the catalase is present in an amount up to about 150 units/ml.

10. The stabilized calibrator solution of claim 7 sealed in a reclosable, airtight container.

11. A method for stabilizing an aqueous bilirubin calibrator solution comprising:

preparing a matrix containing:

between about 0.1 and 0.53 weight percent glucose;

a substrate for an oxygen transfer system which reduces oxygen to water at between about 5 and 30 mM; and

between about 1 and 10 weight percent albumin;

adjusting the pH of the matrix to from about 7.4 to about 8.1;

adding into the matrix sterile membrane fragments derived from bacteria having membranes containing said oxygen transfer system so as to obtain between about 0.3 and 0.6 units/ml of activity and thereafter excluding air from contacting said matrix;

after oxygen is removed from said matrix by the oxygen transfer system, adding into said matrix glucose oxidase so as to obtain from about 10 to 27 units/ml; and

adding into said matrix bilirubin dissolved in 0.1M NaOH so as to obtain between about 1 and 30 mg/dl of bilirubin said dissolved bilirubin being substantially clear.

12. The method of claim 11 wherein the matrix obtained after the addition of bilirubin is sealed in a reclosable, airtight container.

Previous Doc Next Doc Go to Doc#

Inhibitors of Oxidative Phosphorylation

- Several chemicals can block electron transfer in ETS, or transfer of electrons to oxygen. All are strong poisons. Some examples:
  - Carbon monoxide -- combines directly with terminal cytochrome **oxidase**, blocks oxygen attachment
  - Cyanide (CN^-) and **Azide** (N_3^-) bind to cytochrome iron atoms, prevent electron transfer.
  - Antimycin A (an antibiotic) inhibits electron transfer between cyt b and c.

Anaerobic respiration:

- Use of acceptors other than oxygen.
- Most common in bacteria. Most alternative electron acceptors are inorganic molecules, but some organic molecules can serve.
- As with aerobic respiration, **anaerobic** respiration uses ETS, membrane localization, proton gradient, and ATP synthase.
- Processes are of great importance both ecologically and industrially.

Examples of anaerobic respiration:

1. **Nitrate (NO_3^-).**
   - Process called **denitrification**. Also called dissimilative nitrate reduction. Reduced waste products are excreted in significant amounts.
   - Redox potential is + 0.42 v (compared to + 0.82 v for oxygen). So organisms respiring anaerobically gain less energy than with oxygen.
   - Requires new terminal **oxidase** called **nitrate reductase**. Enzyme is repressed by oxygen, synthesis turned on in absence of oxygen.
   - Process can have several steps, proceed in two different directions:
     1. (A) nitrate (NO_3^-) ---> nitrite (NO_2^-) ---> ---> ---> ammonia (NH_3)
     2. (B) nitrate (NO_3^-) ---> nitrite (NO_2^-) ---> nitrous oxide (N_2O) ---> ---> dinitrogen gas (N_2)
   - Second process is major pathway for loss of nitrogen compounds from soil, return of nitrogen to atmosphere.
   - *Pseudomonas* species are common denitrifiers, widespread in soils. When fertilized soils become flooded, oxygen is rapidly depleted, pseudomonads switch to **anaerobic** respiration and can use up soil nitrate, leaving field in unfertile state.
   - Note: Studied this in lab. Media must contain nitrate in addition to nutrients, otherwise won't work. Also, in scavenger hunt at end of course, one target microbe will be *Pseudomonas*, enrichment culture depends on its ability to grown anaerobically using nitrate reduction.
2. **2. Sulfate (SO_4^{2-}).**
   - Process called **sulfate reduction**.
   - Sulfate (SO_4^{2-}) ---> ---> ---> ---> Hydrogen Sulfide (H_2S)
   - Small group of bacteria carry out this reaction; all obligate anaerobes.

- Have unique cytochrome c3.
- Sulfate is common in seawater. Often, H_2S combines with iron, forms insoluble FeS ----> black sediments. Common in estuaries.

3. **3. Carbon dioxide (CO_2).**
    - One of most common inorganic ions.
    - **Methanogens**: most important group of CO_2 reducers. Obligate anaerobes, archaebacteria. Produce methane as waste product.
    - Reaction: $CO_2 + H_2 + H^+ \text{--->} CH_4 + H_2O$
    - Note: reaction also requires Hydrogen gas. Methanogens typically live alongside bacteria that produce hydrogen by fermentation, remove hydrogen as it is made.

Novel stable lipid standards

1. A stable aqueous lipid reference standard composition comprising a substantially pure constituent of known value and a stabilizing amount of at least one antioxidant said composition ranging in pH from about 6.5 to 8.0.

2. The reference composition of claim 1, wherein said reference composition is useful for at least one us selected from the group consisting of calibration, quality control, calibration verification, and assessmen of linearity, and further wherein said composition is useful in a pH range from about 6.5 to 9.0.

3. The reference composition of claim 2, wherein said use comprises a manual, semi-automated, and fully-automated method comprising an instrument for measurement of said constituent.

4. The reference composition of claim 1, wherein said antioxidant is selected from the group consisting of monothioglycerol, fumaric acid, ethylenediaminetetraacetic acid (EDTA), butylated hydroxytoluene (BHT), N-acetyl-L-cysteine, Oxyrase.TM., ascorbic acid, phytic acid dodecasodium salt and 3,3'-thiodipropionic acid (TDPA).

5. The reference composition of claim 1, wherein said stabilizing amount ranges from about 1.1 grams per liter to 18.0 grams per liter.

6. The reference composition of claim 1, wherein said constituent is a substantially pure component of a least one constituent selected from the group consisting of total cholesterol (CHOL), triglycerides (TRIG), low density lipoprotein (LDL), high density lipoprotein (HDL), apolipoprotein A (APO-A), apolipoprotein B (APO-B), apolipoprotein a (Lp(a)), and a subcomponent of an apolipoprotein.

7. The reference composition of claim 6, wherein said subcomponent is at least one subcomponent selected from the group consisting of AII, AIV, B-48, B-100, CI, CII, CIII, D, E1, E2, E3, E4, E5, E6, F G, H, and J.

8. The reference composition of claim 6, wherein said value of CHOL ranges from about 0 to 5000 mg/dL.

9. The reference composition of claim 6, wherein said value of triglycerides ranges from about 0 to 400C mg/dL.

10. The reference composition of claim 6, wherein said value of LDL ranges from about 0 to 5000 mg/dL.

11. The reference composition of claim 6, wherein said value of HDL ranges from about 0 to 1000 mg/dL.

12. The reference composition of claim 6, wherein said value of APO-A ranges from about 0 to 1000 mg/dL.

13. The reference composition of claim 6, wherein said value of APO-B ranges from about 0 to 1000 mg/dL.

14. The reference composition of claim 6, wherein said value of Lp(a) ranges from about 0 to 1200 mg/dL.

15. The reference composition of claim 6, wherein said value of said subcomponent ranges from about 0 to 500 mg/dL.

16. The reference composition of claim 6, said composition further comprising a buffer comprising N-2-hydroxyethylpiperazine-N-2-ethane sulphonic acid (HEPES) such that said composition ranges in pH from about 6.5 to 8.0.

17. The reference composition of claim 6, said composition further comprising a buffer selected from the group consisting of: a buffer comprising N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES) such that said composition ranges in pH from about 6.8 to 8.3; a buffer comprising 3-(N-Morpholino) propanesulfonic acid (MOPS) such that said composition ranges in pH from about 6.8 to 8.3; a buffer comprising N-[Tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid (TES) such that said composition ranges in pH from about 6.8 to 8.3; a buffer comprising Tris(hydroxylmethyl) aminomethane (TRIS) such that said composition ranges in pH from about 6.8 to 8.3; and a buffer comprising Sorenson's Phosphate buffer such that said composition ranges in pH from about 6.8 to 8.3.

18. The reference composition of claim 6, said composition further comprising an amount of sodium azide less than about 0.09% (weight/volume).

19. The reference composition of claim 6, said composition further comprising ProClin.TM. in an amount ranging from about 10 parts per million (ppm) to 100 ppm.

20. The reference composition of claim 6, said composition further comprising Oxyrase.TM. in an amount ranging from about 0.05 U/mL to 0.5 U/mL.

21. A stable control or calibration standard composition comprising a stabilizing amount of 3,3'-thiodipropionic acid, said composition further comprising a predetermined content of substantially pure analyte.

22. A method for producing the reference composition of claim 1, said method comprising mixing a liquid comprising water, with a known value of a substantially pure constituent to produce a mixture and further adding a stabilizing amount of 3,3'-thiodipropionic acid to said mixture.

23. A method for producing a stable control or calibration standard composition, said method comprising mixing an aqueous solution with a stabilizing amount of 3,3'-thiodipropionic acid, and further comprising adding a predetermined amount of an essentially pure analyte.

24. The method of claim 23, wherein said aqueous solution is selected from the group consisting of water, plasma, serum, a solution comprising bovine serum albumin, a solution comprising human serum

albumin, and absolution comprising a Good's Buffer.

25. The method of claim 23, said method further comprising adding to said mixture an effective amount of an antimicrobial.

26. The method of claim 23, wherein said analyte is at least one analyte selected from the group consisting of total cholesterol (CHOL), triglycerides (TRIG), low density lipoprotein (LDL), high density lipoprotein (HDL), apolipoprotein A (APO-A), apolipoprotein B (APO-B), Lp(a) and a subcomponent of an apolipoprotein.

27. A method for calibration, quality control, calibration verification, or assessment of linearity of an instrument wherein said instrument is adapted to determine an amount of a constituent in a test solution, said method comprising subjecting said instrument to calibration, quality control, calibration verificatior or assessment of linearity with the reference composition of claim 1.

28. The method of claim 27, wherein said determination provides information related to the condition of a living subject, said condition being selected from the group consisting of a cholesterol level, a triglyceride level, a HDL level, a LDL level, and APO-A level, and APO-B level, a Lp(a) levels, in support of the diagnosis, treatment, and/or monitoring of, but not limited to, coronary artery disease, atherosclerosis, diabetes mellitus, various hyperlipidemias (either genetic or induced), genetic abnormalities of lipoprotein metabolism, obstructive liver disease, renal insufficiency, hypothyroidism.

29. A method for calibration, quality control, calibration verification, or assessment of linearity of an instrument adapted to determine the amount of an analyte in a test solution, said method comprising subjecting said instrument to calibration, quality control, calibration verification, or assessment of linearity with the composition of claim 1.

30. The method of claim 27, wherein said determination provides information related to the condition of a living subject, said condition being selected from the group consisting of a cholesterol level, a triglyceride level, a HDL level, a LDL level, and APO-A level, and APO-B level, a Lp(a) levels, in support of the diagnosis, treatment, and/or monitoring of, but not limited to, coronary artery disease, atherosclerosis, diabetes mellitus, various hyperlipidemias (either genetic or induced), genetic abnormalities of lipoprotein metabolism, obstructive liver disease, renal insufficiency, hypothyroidism.

31. A kit for calibration, quality control, calibration verification, or calibration of linearity of an instrument adapted to determine the amount of a constituent in a test solution, said kit comprising a composition comprising a known amount of said constituent and a stabilizing amount of 3,3'-thiodipropionic acid, said kit further comprising an applicator and an instructional material for the use o said kit.

32. The kit of claim 31, wherein said composition comprises about 4.0 g/L TDPA, about 0.9 g/L sodium azide, about 40 ppm ProClin.TM., and further comprises HEPES buffer at about pH 7.4, wherein the pH of said composition ranges from about pH 7.2 to 7.6.

33. The kit of claim 31, wherein said kit comprises at least one composition selected from the group consisting of: a) a composition comprising about 10 mg/dL total cholesterol, about 5 mg/dL HDL, about 0 mg/dL triglycerides, about 5 mg/dL LDL, about 15.6 mg/dL APO-A, and about 3.3 mg/dL APO-B; b) a composition comprising about 200 mg/dL total cholesterol, about 37.5 mg/dL HDL, about 250 mg/dL triglycerides, about 162.5 mg/dL LDL, about 117.2 mg/dL APO-A, and about 106.9 mg/dL APO-B; c) a composition comprising about 400 mg/dL total cholesterol, about 115 mg/dL HDL, about 500 mg/dL triglycerides, about 285 mg/dL LDL, about 359.4 mg/dL APO-A, and about 187.5 mg/dL APO-B; d) a

composition comprising about 600 mg/dL total cholesterol, about 177.5 mg/dL HDL, about 750 mg/dL triglycerides, about 422.5 mg/dL LDL, about 554.7 mg/dL APO-A, and about 287.0 mg/dL APO-B; and e) a composition comprising about 800 mg/dL total cholesterol, about 200 mg/dL HDL, about 1000 mg/dL triglycerides, about 600 mg/dL LDL, about 625 mg/dL APO-A, and about 394.7 mg/dL APO-B.

34. The kit of claim 31, wherein said kit comprises at least one composition selected from the group consisting of: a) a composition comprising about 0 mg/dL total cholesterol, about 0 mg/dL HDL, about (mg/dL triglycerides, about 0 mg/dL LDL, about 0 mg/dL APO-A, and about 0 mg/dL APO-B; and b) a composition comprising about 200 mg/dL total cholesterol, about 60 mg/dL HDL, about 250 mg/dL triglycerides, about 140 mg/dL LDL, about 188 mg/dL APO-A, and about 92 mg/dL APO-B.

35. The kit of claim 31, wherein said kit comprises at least one composition selected from the group consisting of: a) a composition comprising about 100 mg/dL total cholesterol, about 20 mg/dL HDL, about 90 mg/dL triglycerides, about 80 mg/dL LDL, about 63 mg/dL APO-A, and about 53 mg/dL APO B; b) a composition comprising about 250 mg/dL total cholesterol, about 50 mg/dL HDL, about 150 mg/dL triglycerides, about 200 mg/dL LDL, about 156 mg/dL APO-A, and about 132 mg/dL APO-B; and c) a composition comprising about 500 mg/dL total cholesterol, about 100 mg/dL HDL, about 250 mg/dL triglycerides, about 400 mg/dL LDL, about 313 mg/dL APO-A, and about 263 mg/dL APO-B.

36. The kit of claim 31, wherein said kit comprises at least one composition selected from the group consisting of: a) a composition comprising about 100 mg/dL total cholesterol, about 25 mg/dL HDL, about 50 mg/dL triglycerides, about 75 mg/dL LDL, about 78 mg/dL APO-A, and about 49 mg/dL APO B; b) a composition comprising about 275 mg/dL total cholesterol, about 48.75 mg/dL HDL, about 287.: mg/dL triglycerides, about 226.25 mg/dL LDL, about 152.25 mg/dL APO-A, and about 150 mg/dL APO-B;.c) a composition comprising about 450 mg/dL total cholesterol, about 72.5 mg/dL HDL, about 525 mg/dL triglycerides, about 377.5 mg/dL LDL, about 226.5 mg/dL APO-A, and about 251 mg/dL APO-B; d) a composition comprising about 625 mg/dL total cholesterol, about 96.25 mg/dL HDL, abou 762.5 mg/dL triglycerides, about 528.75 mg/dL LDL, about 300.75 mg/dL APO-A, and about 352 mg/dL APO-B; and e) a composition comprising about 800 mg/dL total cholesterol, about 120 mg/dL HDL, about 1000 mg/dL triglycerides, about 680 mg/dL LDL, about 375 mg/dL APO-A, and about 453 mg/dL APO-B.

37. The kit of claim 31, wherein said kit comprises at least one composition selected from the group consisting of: a) a composition comprising about 0 mg/dL total cholesterol, about 0 mg/dL HDL, about (mg/dL triglycerides, about 0 mg/dL LDL, about 0 mg/dL APO-A, and about 0 mg/dL APO-B; and b) a composition comprising greater than about 240 mg/dL total cholesterol, less than about 35 mg/dL HDL, about 100 mg/dL triglycerides, greater than about 190 mg/dL LDL, greater than about 120 mg/dL APO-A, and less than about 120 mg/dL APO-B.

38. The kit of claim 31, wherein said kit comprises at least one composition selected from the group consisting of: a) a composition comprising about 0 mg/dL total cholesterol, about 0 mg/dL HDL, about 100 mg/dL triglycerides, about 0 mg/dL LDL, about 0 mg/dL APO-A, and about 0 mg/dL APO-B; and b) a composition comprising greater than about 240 mg/dL total cholesterol, less than about 45 mg/dL HDL, about 100 mg/dL triglycerides, greater than about 190 mg/dL LDL, greater than about 120 mg/dL APO-A, and less than about 120 mg/dL APO-B.

Brief Patent Description - Full Patent Description - **Patent Claims**
Click on the above for other options relating to this Novel stable lipid standards patent application.
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Previous Patent Application:
Method and apparatus for determining one or more operating parameters for a microfluidic circuit
Next Patent Application:
Quantitative determination of atmospheric hydroperoxyl radical
Industry Class:
Chemistry: analytical and immunological testing

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0029] The preferred biocatalytic oxygen reducing agent (see "A Novel Approach to the Growth of Anaerobic Microorganisms" of Adler, et al., Biotechnol. Bioegn. Symp. 11, J. Wiley & Sons, New York, 1981, p. 533 and U.S. Pat. No. 4,476,224 issued Oct. 9, 1984 to Adler entitled "Material and Method for Promoting the Growth of Anaerobic Bacteria") utilized in the invention is comprised of oxygen scavenging membrane fragments which contain an electron transport system which reduces oxygen to water in the presence of a hydrogen donor. These oxygen scavenging membrane fragments can be derived from the cytoplasmic membranes of bacteria (U.S. Pat. No. 4,476,224) and/or from the membranes of mitochondrial organelles of a large number of higher non-bacterial organisms. Other known biocatalytic oxygen reducing agents such as glucose oxidase, alcohol oxidase, etc. can also be utilized.

Abstract

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original article

Bacterial microflora of normal and telangiectatic livers in cattle

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Objective—To identify potential bacterial pathogens in normal and telangiectatic livers of mature cattle at slaughter and to identify consumer risk associated with hepatic telangiectasia.

Sample Population—50 normal livers and 50 severely telangiectatic livers.

Procedure—Normal and telangiectatic livers were collected at slaughter for aerobic and anaerobic bacterial culture. Isolates were identified, and patterns of isolation were analyzed. Histologic examination of all livers was performed.

Results—Human pathogens isolated from normal and telangiectatic livers included *Escherichia coli* O157:H7 and group-D streptococci. Most livers in both groups contained bacteria in low numbers; however, more normal livers yielded negative culture results. More group-D streptococci were isolated from the right lobes of telangiectatic livers than from the left lobes, and more gram-negative anaerobic bacteria were isolated from left lobes of telangiectatic livers than from right lobes. All telangiectatic lesions were free of fibrosis, active necrotizing processes, and inflammation.

Conclusions and Clinical Relevance—The USDA regulation condemning telangiectatic livers is justified insofar as these livers contain more bacteria than normal livers do; however, normal livers contain similar species of microflora. Development of telangiectasia could not be linked to an infectious process. The finding of *E coli* O157:H7 in bovine livers suggests that information regarding bacterial content of other offal and muscle may identify sources of this and other potential foodborne pathogens and assist in establishing critical control points for the meat industry. (*J Am Vet Med Assoc* 2001;219:36–39)

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Public Veterinary Medicine: Food Safety
File Format: PDF/Adobe Acrobat
oxyrase are used as enrichment media for recovery of aerobic ... blood agar

L6: Entry 13 of 17 File: USPT Mar 20, 2001

DOCUMENT-IDENTIFIER: US 6204051 B1
TITLE: Apparatus and method for growing anaerobic microorganisms

Brief Summary Text (6):
Control of the environment is necessary for control of microbial growth. In particular, control of oxygen content in the immediate environment is crucial for microbial growth. Microorganisms can be divided into groups based on their need for, and tolerance of, oxygen. There are those that require oxygen to grow. These are "aerobes". Some microorganisms are able to grow with or without oxygen. These are "facultative anaerobes". Another group of microorganisms can grow only in the presence of very low levels of oxygen. These are the "microaerophiles". Finally, some microorganisms can not tolerate oxygen. They are inhibited by it or may be killed by it. These are the "anaerobes".

Brief Summary Text (16):
However, a number of drawbacks exist in the use of the Brewer Lid. The capacity and the rate for oxygen removal is limited by the sensitivity of the microorganism to the chemical reducing agent in the medium (see "Mechanism of Growth Inhibitory Effect of cysteine on Escherichia coli." of Kari, et al., J. Gen. Microbiol., 68, 1971, p. 349 and "Methods for General and Molecular Bacteriology", Editor: Gerherdt, American Society for Microbiology, 1994, p. 146.). Moreover, the lid is made of heavy glass and is expensive. It is available today (Kimble Glass Company, Vineland, N.J.), but is not widely used because of serious limitations that include cost, handling difficulties, and poor response of anaerobic microorganisms.

Brief Summary Text (37):
Further, the culture dish, i.e., "OxyDish.TM.", of the present invention, is designed in certain embodiments so that it can be stacked in a stable configuration. The dish top has a stacking ring that interlocks with the adjacent dish top below it. The dish bottom, when the assembled dish is inverted and placed in a sealed position, rests (i.e., nests) between the two adjacent dish tops. The functionality of the dish to establish and maintain an anaerobic environment is preserved and protected in the stack. The stackability of the culture dish increases the efficient use of incubator space. Stackability is also important for the mechanized filling of these dishes and shipment of dishes or of finished pre-made, plates to the microbiologist or end user.

Detailed Description Text (58):
Nutrient agar is supplemented with sodium formate (15 mM), sodium succinate (30 mM), sodium lactate (45 mM) and cysteine (0.025 g/100 ml). A biocatalytic oxygen reducing agent, EC-Oxyrase.RTM. (Oxyrase, Inc., Mansfield, Ohio) is added to cooled (45.degree. C. to 50.degree. C.) but molten sterile medium to give a final concentration of 5 units/ml. 20 ml of the above mixture is soon introduced into the bottom part of a culture dish, i.e., "OxyDish.TM.". The top part of the culture dish, is placed over the filled bottom part to prevent contaminants from entering the dish. The agar in the bottom part cools to ambient temperature and solidifies. The covered dish is left standing to permit excess moisture to escape. At this point the dish may be sealed by inverting it to bring the agar surface in the dish bottom into contact with the ring inside the dish top.

PDF] [Public Veterinary Medicine: Food Safety]
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oxyrase are used as enrichment media for recovery of aerobic ... blood agar with sodium **azide**,. e. and salt mannitol ...
avmajournals.avma.org/doi/abs/10.2460/javma.2001.219.36 - Similar pages

L6: Entry 7 of 17 File: PGPB Apr 18, 2002

DOCUMENT-IDENTIFIER: US 20020045245 A1
TITLE: Apparatus and method for growing anaerobic microorganisms

Summary of Invention Paragraph:
[0006] Control of the environment is necessary for control of microbial growth. In particular, control of oxygen content in the immediate environment is crucial for microbial growth. Microorganisms can be divided into groups based on their need for, and tolerance of, oxygen. There are those that require oxygen to grow. These are "aerobes". Some microorganisms are able to grow with or without oxygen. These are "facultative anaerobes". Another group of microorganisms can grow only in the presence of very low levels of oxygen. These are the "microaerophiles". Finally, some microorganisms can not tolerate oxygen. They are inhibited by it or may be killed by it. These are the "anaerobes".

Summary of Invention Paragraph:
[0016] However, a number of drawbacks exist in the use of the Brewer Lid. The capacity and the rate for oxygen removal is limited by the sensitivity of the microorganism to the chemical reducing agent in the medium (see "Mechanism of Growth Inhibitory Effect of cysteine on Escherichia coli." of Kari, et al., J. Gen. Microbiol., 68, 1971, p. 349 and "Methods for General and Molecular Bacteriology", Editor: Gerherdt, American Society for Microbiology, 1994, p. 146.). Moreover, the lid is made of heavy glass and is expensive. It is available today (Kimble Glass Company, Vineland, N.J.), but is not widely used because of serious limitations that include cost, handling difficulties, and poor response of anaerobic microorganisms.

Summary of Invention Paragraph:
[0039] Further, the culture dish, i.e., "OxyDish.TM.", of the present invention, is designed in certain embodiments so that it can be stacked in a stable configuration. The dish top has a stacking ring that interlocks with the adjacent dish top below it. The dish bottom, when the assembled dish is inverted and placed in a sealed position, rests (i.e., nests) between the two adjacent dish tops. The functionality of the dish to establish and maintain an anaerobic environment is preserved and protected in the stack. The stackability of the culture dish increases the efficient use of incubator space. Stackability is also important for the mechanized filling of these dishes and shipment of dishes or of finished pre-made, plates to the microbiologist or end user.

Detail Description Paragraph:
[0113] Nutrient agar is supplemented with sodium formate (15 mM), sodium succinate (30 mM), sodium lactate (45 mM) and cysteine (0.025 g/100 ml). A biocatalytic oxygen reducing agent, EC-Oxyrase.RTM. (Oxyrase, Inc., Mansfield, Ohio) is added to cooled (45.degree. C. to 50.degree. C.) but molten sterile medium to give a final concentration of 5 units/ml. 20 ml of the above mixture is soon introduced into the bottom part of a culture dish, i.e., "OxyDish.TM.". The top part of the culture dish, is placed over the filled bottom part to prevent contaminants from entering the dish. The agar in the bottom part cools to ambient temperature and solidifies. The covered dish is left standing to permit excess moisture to escape. At this point the dish may be sealed by inverting it to bring the agar surface in the dish bottom into contact with the ring inside the dish top.

L6: Entry 7 of 17 File: PGPB Apr 18, 2002

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TITLE: Apparatus and method for growing anaerobic microorganisms

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INVENTOR-INFORMATION:

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Oxyrase, Inc.				

APPL-NO: 09/812204 [PALM]
DATE FILED: March 19, 2001

RELATED-US-APPL-DATA:
Application 09/812204 is a continuation-of US application 09/321812, filed May 28, 1999, UNKNOWN

INT-CL-PUBLISHED: [07] C12 M 1/22

US-CL-PUBLISHED: 435/305.3; 435/303.2
US-CL-CURRENT: 435/305.3; 435/303.2

REPRESENTATIVE-FIGURES: 1

ABSTRACT:

An apparatus for growing anaerobic microorganisms is provided having a dish top that contains a sealing ring upon which the media surface in the dish bottom rests when the apparatus is inverted. The contact between the sealing ring and the media surface forms a seal that traps the gas in the headspace between the media surface and the inside of the dish top. A oxygen reducing agent can also be incorporated into the media together, in some instances, with a substrate which react with oxygen in the media and with oxygen in the headspace thereby creating an environment suitable for growing anaerobic, microaerophilic and facultative anaerobic microorganisms.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part application of Ser. No. 08/963/664, filed Nov. 3, 1997, now U.S. Patent No., which is a continuation application of Ser. No. 08/237,773, filed May 4, 1994, now U.S. Pat. No. 5,830,746, issued Nov. 3, 1998.

Previous Doc Next Doc Go to Doc#

5,830,746

US005955344A

United States Patent [19]

Copeland et al.

[11] **Patent Number: 5,955,344**

[45] **Date of Patent: *Sep. 21, 1999**

[54] **APPARATUS AND METHOD FOR GROWING ANAEROBIC MICROORGANISMS**

[75] Inventors: **James C. Copeland**, Ashland, Ohio; **Howard I. Adler; Gerald E. Spady**, both of Oak Ridge, Tenn.

[73] Assignee: **Oxyrase, Inc.**, Mansfield, Ohio

[*] Notice: This patent is subject to a terminal disclaimer.

[21] Appl. No.: **08/963,664**

[22] Filed: **Nov. 3, 1997**

Related U.S. Application Data

[63] Continuation of application No. 08/237,773, May 4, 1994, Pat. No. 5,830,746.

[51] **Int. Cl.**[6] **C12N 1/00**

[52] **U.S. Cl.** **435/243**; 435/395; 435/420; 435/288.3; 435/303.2; 435/305.4; 435/307.1; 435/801

[58] **Field of Search** 435/243, 325, 435/395, 420, 303.2, 305.4, 307.1, 801, 288.3; 422/102

[56] **References Cited**

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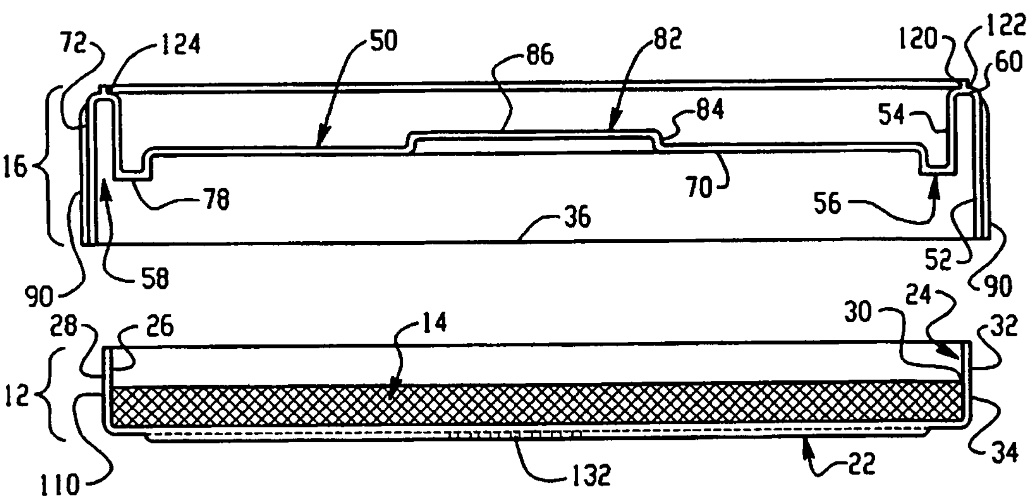
Primary Examiner—David A. Redding

Attorney, Agent, or Firm—Fay, Sharpe, Beall, Fagan, Minnich & McKee, LLP

[57] **ABSTRACT**

An apparatus for growing anaerobic microorganisms is provided having a dish top that contains a sealing ring upon which the media surface in the dish bottom rests when the apparatus is inverted. The contact between the sealing ring and the media surface forms a seal that traps the gas in the headspace between the media surface and the inside of the dish top. A oxygen reducing agent can also be incorporated into the media together, in some instances, with a substrate which react with oxygen in the media and with oxygen in the headspace thereby creating an environment suitable for growing anaerobic, microaerophilic and facultative anaerobic microorganisms.

30 Claims, 6 Drawing Sheets



Microbe	Standard Method	OxyDish Method
Clostridium dificil	1+	3+
Clostridium perfringens	4+	4+
Clostridium cadaveris	1+	3+
Bacteroides rhetaiotaomicron	3+	4+
Bacteroides distasonis	3+	4+
Fusobacterium varium	2+	4+
Fusobacterium mortiferum	2+	4+
Fusobacterium necrophorum	1+	3+
Peptostreptococcus magnus	2+	3+
Peptostreptococcus anaerobius	1+	3+
Peptostreptococcus negra	1+	3+
Bifidobacterium breve	1+	3+
Prevotella intermedia	2+	3+

The invention has been described with reference to the preferred embodiments. Obviously, modifications and alterations will occur to others upon reading and understanding the preceding detailed description. It is intended that the invention be construed as including all such alterations and modifications insofar as they come within the scope of the claims and the equivalents thereof.

What is claimed:

1. An apparatus for enumeration and cultivation of anaerobes, microaerophiles, and facultatively anaerobic microorganisms comprising:

a first dish component having a base and side wall extending therefrom to define a cavity that is adapted to receive a culture medium therein; and,

a second dish component having a wall and side wall extending therefrom that receives the first dish component, the second dish component further comprising a seal surface that faces the first dish component when assembled, the seal surface disposed at a predetermined dimension so that it is spaced from the culture medium in the first orientation and engages the culture medium in the second orientation to define (i) a non-sealed assembly in a first orientation of the dish components and (ii) a closed headspace between the dish components in a second orientation.

2. The apparatus as defined in claim 1, wherein the side wall of the second dish component extends from its wall a greater dimension than the side wall of the first dish component extends from its base.

3. The apparatus as defined in claim 1, wherein the first dish component nests within the second dish component.

4. The apparatus as defined in claim 1, wherein the side wall of the first dish component includes strengthening ribs for increasing the rigidity thereof.

5. The apparatus as defined in claim 1, wherein the side wall of the second dish component includes strengthening ribs for increasing the rigidity thereof.

6. The apparatus as defined in claim 1, wherein the side walls of the first and second dish components include circumferentially spaced ribs for increasing the rigidity of the side walls.

7. The apparatus as defined in claim 1, wherein the side wall of the first dish component includes at least one recess to aid in separation of the first and second dish components.

8. An apparatus for enumeration and cultivation of anaerobes, microaerophiles, and facultatively anaerobic microorganisms comprising:

a first dish component having a base and side wall extending therefrom to define a cavity; and,

a second dish component having a wall and side wall extending therefrom that receives the first dish component, the side wall of the second dish component extending from its wall a greater dimension than the side wall of the first dish component extends from its base, to define (i) a non-sealed assembly in an assembled, first orientation of the dish components and (ii) a closed headspace between the dish components in an assembled, second orientation.

9. The apparatus as defined in claim 1, wherein the second dish component includes a circular rib protruding outwardly from the wall in a direction generally opposite that of the side wall.

10. The apparatus as defined in claim 9, wherein the rib is spaced radially inward from a peripheral edge of the second dish component and is adapted to receive a side wall of an adjacent second dish component when placed in stacked relation.

11. The apparatus as defined in claim 9, wherein the rib is dimensioned to receive at least a portion of a base of an adjacent first dish component when disposed in stacked relation and in the first orientation of the dish components.

12. A method of cultivating and enumerating anaerobes, microaerophiles and facultatively anaerobic microorganisms comprising the steps of:

providing first and second dish components that cooperate to define a cavity adapted to receive a medium

positioning the first and second dish components in an assembled first orientation; and

altering the position of the first and second dish to an assembled second orientation for cultivating microorganisms, defining a sealed headspace between the medium and one of the dish components for cultivating microorganisms.

13. A culture dish for growing anaerobic microorganisms comprising a dish bottom for receiving a solidifiable culture media; and, a dish cover adapted to be placed over said dish bottom, said dish cover having a seal ring spaced from the media surface of the dish bottom when placed in an upright position and wherein said seal ring comes into contact with the media surface in the dish bottom when the culture dish is inverted to form a seal that traps gas in a headspace between the media surface and the dish cover and wherein an oxygen reducing agent is incorporated into the media which reacts with oxygen in the media and in the headspace thereby removing the oxygen and creating an environment suitable for growing anaerobic, microaerophilic and facultative anaerobic microorganisms.

14. A culture dish of claim 13, wherein the dish cover has a height sufficient to prevent the seal ring from contacting the media surface when the dish cover is placed over the dish bottom after receipt of the culture media.

15. A culture dish of claim 13, wherein said seal ring of the dish cover has a depth of approximately 2 mm to 5 mm, to provide sufficient headspace for growth of the microorganisms when the culture dish is inverted.

16. A culture dish of claim 13, wherein said dish cover has a dome adapted to contain a gas releasing agent.

17. A culture dish of claim 13, wherein said dish cover has a dome designed to contain an anaerobic environment indicator agent.

18. A culture dish of claim 13, wherein said dish cover has at least one cut-out for enhancing handling of the dish bottom from the culture dish.

19. A culture dish of claim 13, wherein said dish cover has a rib to permit stacking of assembled culture dishes in a stable configuration.

20. A culture dish of claim 13, wherein said dish bottom comprises a base and a projecting side wall, the side wall having a height sufficient to rest under the dish cover while preventing contact of the media surface of the dish bottom with the seal ring of the dish cover when the culture dish is placed in the upright position and to allow the media surface to come into contact with the seal ring of the dish cover when the culture dish is placed in the inverted position.

21. A culture dish of claim 20, wherein the dish bottom has a fill line on the side wall thereof to indicate the maximum fill height in the dish bottom.

22. A culture dish of claim 13, wherein the dish bottom contains pores having a diameter of approximately 0.1 mm to 0.4 mm to provide for evaporation of moisture from the media contained therein so as to prevent moisture build-up inside the headspace.

23. A culture dish of claim 13, wherein the dish cover is formed from one of polystyrene, polystyrene-acrylonitrile, and polycarbonate.

24. A culture dish of claim 13, wherein the dish bottom is formed from one of polystyrene, polystyrene-acrylonitrile, and polycarbonate.

25. A culture dish of claim 23, wherein the dish bottom and dish cover are formed from a transparent plastic.

26. A culture dish of claim 13 , wherein the dish cover has a stacking rib that facilitates stacking of the assembled culture dishes, dish cover to dish cover, with the dish bottoms nested between adjacent dish covers.

27. A culture dish of claim 13, wherein the oxygen reducing agent is a biocatalytic oxygen reducing agent.

28. A culture dish of claim 27, wherein the biocatalytic oxygen reducing agent is a membrane fraction obtained from bacterial and mitochondrial sources.

29. A culture dish of claim 28, wherein the biocatalytic oxygen reducing agent comprises of glucose oxidase and catalase.

30. A culture dish of claim 13, wherein the media is an appropriate agar media that supports the growth of anaerobes, microaerophiles, and facultative aerobes.

* * * * *

Clin Chem. 1982 Mar;28(3):556-7. Related Articles, Links

Use of azide to enhance the sensitivity of colorimetric immobilized-glucose-oxidase methods for glucose.

Thompson RQ, Patton CJ, Crouch SR.

Publication Types:

- Letter

PMID: 7067112 [PubMed - indexed for MEDLINE]

DOCUMENT-IDENTIFIER: US 6429008 B1
TITLE: Apparatus and method for growing anaerobic microorganisms

Brief Summary Text (6):
Control of the environment is necessary for control of microbial growth. In particular, control of oxygen content in the immediate environment is crucial for microbial growth. Microorganisms can be divided into groups based on their need for, and tolerance of, oxygen. There are those that require oxygen to grow. These are "aerobes". Some microorganisms are able to grow with or without oxygen. These are "facultative anaerobes". Another group of microorganisms can grow only in the presence of very low levels of oxygen. These are the "microaerophiles". Finally, some microorganisms can not tolerate oxygen. They are inhibited by it or may be killed by it. These are the "anaerobes".

Brief Summary Text (16):
However, a number of drawbacks exist in the use of the Brewer Lid. The capacity and the rate for oxygen removal is limited by the sensitivity of the microorganism to the chemical reducing agent in the medium (see "Mechanism of Growth Inhibitory Effect of cysteine on Escherichia coli." of Kari, et al., J. Gen. Microbiol., 68, 1971, p. 349 and "Methods for General and Molecular Bacteriology", Editor: Gerherdt, American Society for Microbiology, 1994, p. 146.). Moreover, the lid is made of heavy glass and is expensive. It is available today (Kimble Glass Company, Vineland, N.J.), but is not widely used because of serious limitations that include cost, handling difficulties, and poor response of anaerobic microorganisms.

Brief Summary Text (37):
Further, the culture dish, i.e., "OxyDish.TM.", of the present invention, is designed in certain embodiments so that it can be stacked in a stable configuration. The dish top has a stacking ring that interlocks with the adjacent dish top below it. The dish bottom, when the assembled dish is inverted and placed in a sealed position, rests (i.e., nests) between the two adjacent dish tops. The functionality of the dish to establish and maintain an anaerobic environment is preserved and protected in the stack. The stackability of the culture dish increases the efficient use of incubator space. Stackability is also important for the mechanized filling of these dishes and shipment of dishes or of finished pre-made, plates to the microbiologist or end user.

Detailed Description Text (42):
Nutrient agar is supplemented with sodium formate (15 mM), sodium succinate (30 mM), sodium lactate (45 mM) and cysteine (0.025 g/100 ml). A biocatalytic oxygen reducing agent, EC-Oxyrase.RTM. (Oxyrase, Inc., Mansfield, Ohio) is added to cooled (45.degree. C. to 50.degree. C.) but molten sterile medium to give a final concentration of 5 units/ml. 20 ml of the above mixture is soon introduced into the bottom part of a culture dish, i.e., "OxyDish.TM.". The top part of the culture dish, is placed over the filled bottom part to prevent contaminants from entering the dish. The agar in the bottom part cools to ambient temperature and solidifies. The covered dish is left standing to permit excess moisture to escape. At this point the dish may be sealed by inverting it to bring the agar surface in the dish bottom into contact with the ring inside the dish top.

Previous Doc

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(19) United States
(12) Patent Application Publication
(10) Pub. No.: US 2003/0124643 A1
Taintor
(43) Pub. Date: Jul. 3, 2003

(54) METHOD AND KIT FORRAPID CONCURRENT IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY TESTING OF MICROORGANISMS FROM BROTH CULTURE

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(21) Appl. No.: 09/998,638

(22) Filed: Dec. 3, 2001

Publication Classification

(51) Int. Cl.[7] C12Q 1/04; C12Q 1/08
(52) U.S. Cl. 435/40; 435/34

(57) ABSTRACT

A Method and Kit for performing concurrent identification testing and antimicrobial susceptibility testing from broth culture (90) are described. Broth (82) incubation is generally 4 to 6 hrs providing adequate numbers of microorganisms for inoculating a multi-chambered kit plate (80) comprising enriched, differential, selective, differential-selective, single-purpose and susceptibility media. Several dilutions are prepared from the cultured broth, for inoculation of the kit plate (80). The more dilute concentration (140) produces individual colonies of microorganisms, for identification testing. This isolation makes an initial isolation step unnecessary. The heavier concentration dilution (96) provides inoculation for antimicrobial susceptibility tests and other identification tests. In addition, antimicrobial susceptibilities are shown valid even when several different microorganisms coexist in the same test chamber. The method is fast for bacteria, providing identification and susceptibility data in 24 hrs. The kit is complete, except for an incubator and microscope. The method is simple to perform and can be utilized almost anywhere.

generate a set of criteria from the kit results for these and other types of microorganisms. In addition, it is practical to generate additional criteria using additional methods of biochemistry for more definitive identification.

[0290] The process of preserving the kit plates for later use comprise the packaging and storage under a nitrogen atmosphere performed in a glove box in a low permeability bag. Other embodiments would be to package under nitrogen in a Mylar-foil bag for complete protection against oxygen. Another inert gas could be used to package the kit plates also with another type of impermeable bag or container.

[0291] Accordingly, the scope of the invention should be determined not by the embodiments(s) illustrated, but by the appended claims and their legal equivalents.

I claim:

1. A kit for quickly performing a plurality of microbiological test(s) on a broth culture, wherein more than one type of microorganism may exist and comprising:

a. said broth culture, previously inoculated with a microbial sample, providing sufficient numbers of microorganisms for said microbiological test(s) and

b. a kit plate comprising a plurality of test chambers comprising a plurality of identification testing media and antimicrobial susceptibility testing media and

c. antimicrobial impregnated carriers for use with said antimicrobial susceptibility testing media

whereby rapid said microbiological tests, comprising concurrent identification testing and antimicrobial susceptibility testing of one to several microorganism types from said microbial sample, may be performed

2. The kit of claim 1 wherein said broth comprises a rich liquid media sufficient for rapid growth of microorganisms.

3. The kit of claim 1 wherein said broth may be selective for a particular type of microorganism.

4. The kit of claim 1 wherein said broth may support the growth of anaerobic microorganisms.

5. The kit of claim 1 wherein said kit plate comprises a plurality of said test chambers comprising a plurality of solid media

6. The kit of claim 1 wherein said kit plate is polystyrene with a lid of similar composition.

7. The kit of claim 1 wherein said test chambers of said kit plate are rectangular with sides of any convenient dimension

8. The kit of claim 1 wherein said kit plate comprises at least 10 said test chambers comprising selective said identification testing media and said susceptibility testing media.

9. The kit of claim 1 wherein said kit plate comprises at least 10 chambers comprising differential said identification testing media and said susceptibility testing media

10. The kit of claim 1 wherein said kit plate comprises at least 10 chambers comprising differential-selective said identification testing media and said susceptibility testing media

11. The kit of claim 1 wherein said kit plate comprises at least 10 chambers comprising single purpose said identification testing media and said susceptibility testing media

12. The kit of claim 1 wherein said kit plate comprises at least 10 chambers comprising enriched said identification testing media and said susceptibility testing media

13. The kit of claim 1 wherein said kit plate comprises at least 10 chambers comprising a combination of said enriched, said selective, said special purpose, said differential-selective, and said differential identification testing media and said susceptibility testing media.

14. The kit of claim 1 wherein said antimicrobial impregnated carriers a reproduced from standard Kirby-Bauer disk-diffusion antimicrobial disks divided into quarters for placement onto a corner of the susceptibility chamber

15. The kit of claim 1 wherein said antimicrobial impregnated carriers can be constructed from any material that acts as an inert carrier of the antimicrobial agent.

16. A method for quickly performing a plurality of said microbiological test(s) on said broth cultured microbial sample where several different microorganism types may exist and comprising the steps of:

a. providing said broth for rapid cultivation of said microbial sample and

b. providing growth of said microbial sample's microorganisms in said broth culture and providing said kit plate with a plurality of test chambers comprising microbiological testing media and

d. providing inoculation of said kit plate with dilutions from said broth culture and

e. providing incubation of said kit plate for sufficient time to reveal colonies, biochemistries and susceptibilities and

f. providing said microbiological testing on said kit plate comprising said identification testing

and said antimicrobial susceptibility testing which may involve more than one said microorganism type in the same said test chamber and

whereby rapid said microbiological tests comprising concurrent said identification testing and said antimicrobial susceptibility testing of one to several said microorganism types from said microbial sample may be performed.

17. The method of claim 16 wherein said broth is incubated from 4 to 8 hrs from an inoculation of said microbial sample.

18. The method of claim 17 wherein the incubated broth is diluted to several different concentrations and inoculated onto said kit plate where the more dilute inoculation produces individual colonies of said microorganisms for identification.

19. The method of claim 18 wherein said individual colonies are further analyzed to reveal identification of the different types of said microorganisms contained in the same said test chambers.

20. The method of claim 18 wherein the greater concentration dilution produces a lawn of the several said microorganism types within the same said test chambers, and where comprising said susceptibility testing media, these different microorganism types are susceptibility tested.

* * * * *

US006153400A

United States Patent [19]

Matsumura et al.

[11] Patent Number: 6,153,400

[45] Date of Patent: Nov. 28, 2000

[54] **DEVICE AND METHOD FOR MICROBIAL ANTIBIOTIC SUSCEPTIBILITY TESTING**

[75] Inventors: **Paul M. Matsumura**, Cary; **Jones M. Hyman**, Durham; **Scott R. Jeffrey**, Raleigh; **Martin J. Maresch**; **Thurman C. Thorpe**, both of Durham; **William G. Barron**, Bahama, all of N.C.

[73] Assignee: **Akzo Nobel N.V.**, Arnhem, Netherlands

[21] Appl. No.: **09/267,863**

[22] Filed: **Mar. 12, 1999**

[51] **Int. Cl.**^{7} **C12Q 1/18**; C12Q 1/04; C12M 1/22; C12M 1/00

[52] **U.S. Cl.** **435/32**; 435/34; 435/4; 435/305.1; 435/305.2; 435/305.3; 435/289.1; 435/283.1; 435/287.1; 435/288.3; 435/288.4

[58] **Field of Search** 435/32, 34, 4, 435/305.1, 305.2, 305.3, 289.1, 283.1, 287.1, 288.3, 288.4

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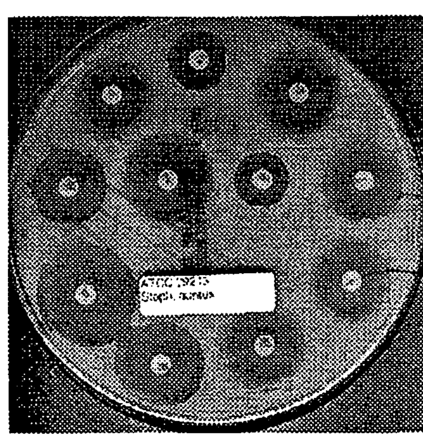
Primary Examiner—Louise N. Leary

Attorney, Agent, or Firm—Gregory R. Muir

[57] **ABSTRACT**

A method and apparatus for performing microbial antibiotic susceptibility testing include disposable, multi-chambered susceptibility plates and an automated plate handler and image acquisition and processing instrument. The susceptibility plates are inoculated with a microorganism (any suitable organism such as bacteria, fungi, protozoa, algae or viruses) and anti-microbial agent(s) are applied such that the microorganism is exposed to a variety of concentrations, or a gradient of each anti-microbial agent. The plates are then placed in the instrument, which monitors and measures the growth (or lack thereof) of the microorganisms. This data is used to determine the susceptibility of the microorganism to the antibiotics. Such a system automates antimicrobial susceptibility testing using solid media and Kirby-Bauer standardized result reporting. The system provides a level of automation previously associated only with broth microdilution testing, while retaining the advantages of the manual disk diffusion test.

49 Claims, 11 Drawing Sheets



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14

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US007018828B1

(12) United States Patent
Taintor

(10) Patent No.: US 7,018,828 B1
(45) Date of Patent: Mar. 28, 2006

(54) MICROBIAL CULTURE MEDIUM CONTAINING AGAR AND IOTA CARRAGEENAN

(76) Inventor: Read Taintor, 98 Mason La., North Salt Lake, UT (US) 84054

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 325 days.

(21) Appl. No.: 10/462,131

(22) Filed: Jun. 16, 2003

Related U.S. Application Data

(63) Continuation-in-part of application No. 10/036,042, filed on Nov. 9, 2001, now abandoned.

(51) Int. Cl.
C12N 1/00 (2006.01)
C12N 1/14 (2006.01)
C12N 1/16 (2006.01)
C12N 1/18 (2006.01)
C12N 1/20 (2006.01)

(52) U.S. Cl. 435/253.6; 435/243; 435/255.21; 435/255.7; 435/256.8

(58) Field of Classification Search 435/243, 435/253.6, 255.21, 255.7, 256.8
See application file for complete search history.

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Webpage, Carrageenan information, 2 pages.
Webpage, Product Information, Carrageenan, 1 page.
Webpage, Carrageenans, 5 pages.
Webpage, GENU Carrageenan, 12 pages.
Webpage, "Carrageenan: Technical Information," CEAMSA, 3 pages.
Webpage, Definitions, 10 pages.
Webpage, FMC Marine Colloids-Carrageenan, FMC BioPolymer, 3 pages.
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Watson, N, et al., "Substitute for Agar in Solid Media for Common Usages in Microbiology," Appl. Environ. Microbiol. Apr. 1976, 31 (4) 509–13.

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Primary Examiner—David M. Naff
Assistant Examiner—Deborah K. Ware
(74) *Attorney, Agent, or Firm*—Madson & Metcalf

(57) ABSTRACT

Media and kits are disclosed for use in processes requiring microbial culture. More specifically, the invention provides carrageenan-stabilized agar-based microbial culture media and kits constructed using the media. The media and kits of the invention may allow the construction of kits with increased shelf stability and useful life. Further, the media and kits of the invention may be used in kits and methods useful in the manual determination of the type of infection present in a specimen in periods of about 24 hours. The stabilized culture media of the invention are useful in a broad variety of applications. In an embodiment, the media contains both an agar medium and iota carrageenan.

39 Claims, 28 Drawing Sheets

(1 of 28 Drawing Sheet(s) Filed in Color)

gens and substrates could have their shelf and open vial life extended by this invention. The particular substance would place limits on the substrate concentrations of Table 1 and any buffer system which may be necessary.

A stabilized anoxic solution containing an oxygen labile reagent could be prepared as in Example 1 which would comprise the substrate of Table 1. Additionally the solution could further comprise BHT or BHA from 0.001 to 0.04%, buffer such as bistrispropane with adequate strength to maintain a pH compatible with the system, a detergent and organic solvent as required for BHT micelle formation. Low levels of glucose oxidase are generally adequate since the substrate turnover is very high, however high levels may to lead greater stability. The absorbance of this yellow compound may be a limiting factor in spectral applications.

Some enzymes and organic and inorganic chemicals are stabilizing by substances that are reducing in nature. Cysteine and ascorbic acid, for example, when used in this fashion are of limited value since they are themselves subject to oxidation. In a matrix such as described in the above examples these oxygen labile substances could be stabilized so that they in turn could function as stabilizer.

In the case of enzyme reagents strong chelators may inactivate the enzyme by chelation of a metallic coenzyme. In such a case the gluconic acid generation may be limited by limiting the glucose substrate. Degassing, pre-treating with the membrane fragment enzyme system and then addition of glucose oxidase may also limit the generation of gluconic acid.

It will be appreciated by one skilled in the art that the embodiments described and the alternative embodiments presented are intended as examples rather than as limitations. Thus, the description of the invention is not intended to limit the invention to the particular embodiments disclosed, but it is intended to encompass all equivalents and subject matter within the spirit and scope of the invention as described above and as set forth in the following claims.

We claim:

1. A stabilized aqueous bilirubin calibrator solution comprising:
- between about 1.0 and 30 mg/dl of bilirubin;
- between about 0.9 and 1.1 weight percent ethanol;
- between about 5 and 6 weight percent polyoxyethylene 23 lauryl ether;
- between about 0.0016 and 0.024 weight percent butylated hydroxy toluene;
- bistrispropane at about 0.1M;
- between about 0.1 and 0.53 weight percent gluconic acid generated in situ from glucose;
- between about 4 and 6 weight percent albumin;
- d-lactate hydrogen donor at between about 3 and 20 mM; and
- between about 0.3 and 0.6 units/ml sterile membrane fragments derived from bacteria having membranes containing an oxygen transfer system which reduces oxygen to water;
- wherein said stabilized aqueous bilirubin calibrator solution has a pH from about 7.7 to about 7.85 and has a storage stability of at least six months at from about 2° to about 8° C. and at least four days at 45° C., said calibrator solution further having an open vial stability of at least three weeks at from about 2° to about 8° C.

2. The bilirubin calibrator solution of claim 1 which further comprises catalase.

3. The bilirubin calibrator solution of claim 2 wherein the catalase is present in an amount up to about 150 units/ml.

4. A method for stabilizing an aqueous bilirubin calibrator solution comprising:
- preparing a matrix containing:
- between about 0.9 and 1.1 weight percent ethanol;
- between about 5 and 6.4 weight percent polyoxyethylene 23 lauryl ether;
- bistrispropane at about 0.1M;
- between about 0.1 and 0.53 weight percent glucose;
- between about 0.0016 and 0.024 weight percent butylated hydroxy toluene;
- d-lactate hydrogen donor at between about 5 and 30 mM; and
- between about 1 and 10 weight percent albumin;
- adjusting the pH of the matrix to from about 7.7 to about 7.85;
- adding into the matrix sterile membrane fragments derived from bacteria having membranes containing an oxygen transfer system which reduces oxygen to water so as to obtain between about 0.3 and 0.6 units/ml of activity and thereafter excluding air from contacting said matrix;
- adding to the air excluded matrix catalase in an amount up to about 150 units/ml;
- after oxygen is removed from said matrix by the oxygen transfer system, adding into said matrix sufficient glucose oxidase to obtain between about 10 and 27 units/ml;
- adding into said matrix bilirubin dissolved in 0.1M NaOH so as to obtain between about 1 and 30 mg/dl of bilirubin in a bilirubin containing matrix, said dissolved bilirubin being substantially clear; and
- sealing said bilirubin containing matrix in a reclosable, air tight container.

5. A stabilized aqueous bilirubin calibrator solution comprising:
- between about 1.0 and 30 mg/dl bilirubin;
- bistrispropane at about 0.1M;
- between about 0.1 and 0.53 weight percent gluconic acid generated in situ from glucose;
- between about 4 to about 6 weight percent albumin;
- d-lactate hydrogen donor at between about 3 and 20 mM; and
- between about 0.3 and 0.6 units/ml sterile membrane fragments derived from bacteria having membranes containing an oxygen transfer system which reduces oxygen to water;
- wherein said stabilized bilirubin calibrator solution has a pH from about 7.7 to about 7.8 and has a storage stability of at least six months at from about 2° to about 8° C. and at least four days at about 45° C., said calibrator solution further having an open vial stability of at least three weeks at from about 2° to about 8° C.

6. The bilirubin calibrator solution of claim 5 which further comprises up to about 0.024 weight percent butylated hydroxy toluene, from about 0.95 to about 1.0 weight percent ethanol, and from about 5 to about 6.4 weight percent polyoxyethylene 23 lauryl ether.

7. A stabilized aqueous bilirubin calibrator solution comprising:
- between about 1.0 and 30 mg/dl bilirubin;
- between about 0.1 and 0.53 weight percent gluconic acid generated in situ from glucose;
- between about 1 and 10 percent albumin;

between 0.3 and 0.6 units/ml sterile membrane fragments derived from bacteria having membranes containing an oxygen transfer system which reduces oxygen to water; and

a substrate for said oxygen transfer system;

wherein said stabilized bilirubin calibrator solution has a pH from about 7.4 to about 8.1 and has a storage stability of at least six months at from about 2° to about 8° C. and at least four days at about 45° C., said calibrator solution further having an open vial stability of at least three weeks at from about 2° to about 8° C.

8. The bilirubin calibrator solution of claim 7 which further comprises catalase.

9. The bilirubin calibrator solution of claim 8 wherein the catalase is present in an amount up to about 150 units/ml.

10. The stabilized calibrator solution of claim 7 sealed in a reclosable, airtight container.

11. A method for stabilizing an aqueous bilirubin calibrator solution comprising:

preparing a matrix containing:

between about 0.1 and 0.53 weight percent glucose;

a substrate for an oxygen transfer system which reduces oxygen to water at between about 5 and 30 mM; and

between about 1 and 10 weight percent albumin;

adjusting the pH of the matrix to from about 7.4 to about 8.1;

adding into the matrix sterile membrane fragments derived from bacteria having membranes containing said oxygen transfer system so as to obtain between about 0.3 and 0.6 units/ml of activity and thereafter excluding air from contacting said matrix;

after oxygen is removed from said matrix by the oxygen transfer system, adding into said matrix glucose oxidase so as to obtain from about 10 to 27 units/ml; and

adding into said matrix bilirubin dissolved in 0.1M NaOH so as to obtain between about 1 and 30 mg/dl of bilirubin said dissolved bilirubin being substantially clear.

12. The method of claim 11 wherein the matrix obtained after the addition of bilirubin is sealed in a reclosable, airtight container.

* * * * *

4,476,224

US 20020045245A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2002/0045245 A1**

Copeland et al. (43) **Pub. Date: Apr. 18, 2002**

(54) **APPARATUS AND METHOD FOR GROWING ANAEROBIC MICROORGANISMS**

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(73) Assignee: **Oxyrase, Inc.**

(21) Appl. No.: **09/812,204**

(22) Filed: **Mar. 19, 2001**

Related U.S. Application Data

(63) Continuation of application No. 09/321,812, filed on May 28, 1999.

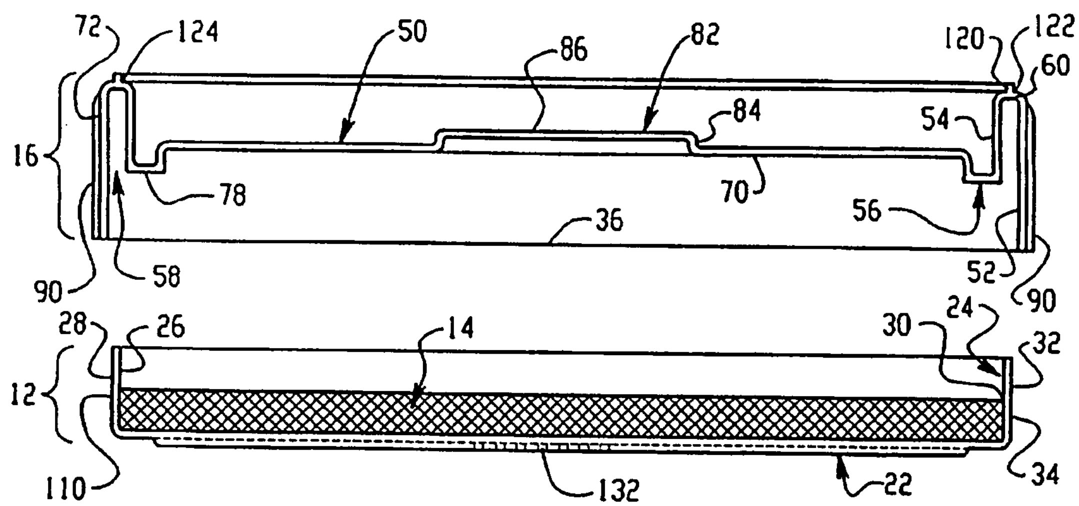
Publication Classification

(51) **Int. Cl.**[7] **C12M 1/22**

(52) **U.S. Cl.** **435/305.3**; 435/303.2

(57) **ABSTRACT**

An apparatus for growing anaerobic microorganisms is provided having a dish top that contains a sealing ring upon which the media surface in the dish bottom rests when the apparatus is inverted. The contact between the sealing ring and the media surface forms a seal that traps the gas in the headspace between the media surface and the inside of the dish top. A oxygen reducing agent can also be incorporated into the media together, in some instances, with a substrate which react with oxygen in the media and with oxygen in the headspace thereby creating an environment suitable for growing anaerobic, microaerophilic and facultative anaerobic microorganisms.



DOCUMENT-IDENTIFIER: US 20020045245 A1
TITLE: Apparatus and method for growing anaerobic microorganisms

Summary of Invention Paragraph:
[0006] Control of the environment is necessary for control of microbial growth. In particular, control of oxygen content in the immediate environment is crucial for microbial growth. Microorganisms can be divided into groups based on their need for, and tolerance of, oxygen. There are those that require oxygen to grow. These are "aerobes". Some microorganisms are able to grow with or without oxygen. These are "facultative anaerobes". Another group of microorganisms can grow only in the presence of very low levels of oxygen. These are the "microaerophiles". Finally, some microorganisms can not tolerate oxygen. They are inhibited by it or may be killed by it. These are the "anaerobes".

Summary of Invention Paragraph:
[0016] However, a number of drawbacks exist in the use of the Brewer Lid. The capacity and the rate for oxygen removal is limited by the sensitivity of the microorganism to the chemical reducing agent in the medium (see "Mechanism of Growth Inhibitory Effect of cysteine on Escherichia coli." of Kari, et al., J. Gen. Microbiol., 68, 1971, p. 349 and "Methods for General and Molecular Bacteriology", Editor: Gerherdt, American Society for Microbiology, 1994, p. 146.). Moreover, the lid is made of heavy glass and is expensive. It is available today (Kimble Glass Company, Vineland, N.J.), but is not widely used because of serious limitations that include cost, handling difficulties, and poor response of anaerobic microorganisms.

Summary of Invention Paragraph:
[0039] Further, the culture dish, i.e., "OxyDish.TM.", of the present invention, is designed in certain embodiments so that it can be stacked in a stable configuration. The dish top has a stacking ring that interlocks with the adjacent dish top below it. The dish bottom, when the assembled dish is inverted and placed in a sealed position, rests (i.e., nests) between the two adjacent dish tops. The functionality of the dish to establish and maintain an anaerobic environment is preserved and protected in the stack. The stackability of the culture dish increases the efficient use of incubator space. Stackability is also important for the mechanized filling of these dishes and shipment of dishes or of finished pre-made, plates to the microbiologist or end user.

Detail Description Paragraph:
[0113] Nutrient agar is supplemented with sodium formate (15 mM), sodium succinate (30 mM), sodium lactate (45 mM) and cysteine (0.025 g/100 ml). A biocatalytic oxygen reducing agent, EC-Oxyrase.RTM. (Oxyrase, Inc., Mansfield, Ohio) is added to cooled (45.degree. C. to 50.degree. C.) but molten sterile medium to give a final concentration of 5 units/ml. 20 ml of the above mixture is soon introduced into the bottom part of a culture dish, i.e., "OxyDish.TM.". The top part of the culture dish, is placed over the filled bottom part to prevent contaminants from entering the dish. The agar in the bottom part cools to ambient temperature and solidifies. The covered dish is left standing to permit excess moisture to escape. At this point the dish may be sealed by inverting it to bring the agar surface in the dish bottom into contact with the ring inside the dish top.

Microbial Ecology in Health and Disease
Publisher: Taylor & Francis
Issue: Volume 11, Number 4 / December 31, 1999
Pages: 208 - 210
URL: Linking Options
DOI: 10.1080/089106099435646

Azoreductase and Nitroreductase in Human Feces: Comparison of Methods to Measure Specific Activities in the Absence of Oxygen

Robert J. Carman, Mary Alice Woodburn

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Abstract:

We measured the specific activity of azoreductase and nitroreductase in eight fresh samples of human feces, using three ways to produce anaerobic assay conditions. A fourth, bench top aerobic assay was also run. Using Oxyrase to produce anaerobic conditions on the open bench gave a significantly higher level of azoreductase than did running the assay entirely in an anaerobic the glove box. Nitroreductase levels were similar in the two assays. Oxyrase and the glove box both gave significantly higher levels of each activity than did either the aerobic bench top or the Anaerobe Lab transfer system. The latter was no improvement over the aerobic, bench top assay. We conclude that Oxyrase is a technically competitive way to conduct anaerobic assays.

The references of this article are secured to subscribers.

Detailed Description Text (139):
For determining the viability indices (Romeo, et al., 1988) of SRB, a late-exponential phase culture (O.D.sub.600 0.16 to 0.19 which corresponded to an initial cell number of 5-9.thrfore.10.sup.4 cells/mL) was exposed to various concentrations of antimicrobials for 1 hour at 30.degree. C. One mL of cells was harvested, washed once in fresh modified Baar's medium to remove cellular debris, and resuspended in 1 mL of fresh modifed Baar's medium supplemented with 10 .mu.L each of Oxyrase (Oxyrase Inc., Mansfeld, Ohio) and 4% sodium sulphide. Aliquots of 450 .mu.L were dispensed in 500 .mu.L sterile eppendorf tubes and appropriate amounts of antimicrobials added and incubated at 30.degree. C. The effectiveness of treatment was determined by the multiple-tube most-probable-number (MPN) fermentation technique. (Anonymous. Multiple-tube fermentation technique for members of the coliform group, pp. 9-45 to 9-51. In A. E. Greenberg, L. S. Clesceri, and A. D. Eaton (eds.), Standard Methods for the Examination of Water and Wastewater, 18 ed. American Public Health Association, American Water Works Association, and Water Pollution Control Federation, New York (1992)) (hereafter, Greenberg, 1992).

L6: Entry 6 of 17 File: PGPB Apr 24, 2003

DOCUMENT-IDENTIFIER: US 20030077232 A1
TITLE: Composition

Summary of Invention Paragraph:
[0002] Alkyl hydroxybenzoates (parabens) are known in the art where the alkyl group is methyl. For example, methyl hydroxybenzoate is mentioned, albeit fleetingly, for use in medicinal and oral care preparations as a preservative (WO 00/09507 and WO 00/69401).

Summary of Invention Paragraph:
[0003] In addition, U.S. Pat. No. 5,094,841 (Fine) discloses the use of heptyl paraben as a preservative in an oral care formulation. However, it also states that the preferred preservatives are methyl and propyl paraben and only ever states that they may be included in small amounts (0.1%) to provide a preservative effect.

Summary of Invention Paragraph:
[0030] preservatives;

Detail Description Paragraph:
[0047] The seed stock of the bacterial strains, E. cloacae, A. naeslundii, S. sanguis (facultative anaerobes) and F. nucleatum and V. parvula (obligate anaerobes) is stored frozen in 1.5 ml aliquots. From the stock, an appropriate dilution of bacteria is added to BHI (dilution 1:500 for E. cloacae; dilution 1:200 for A. naeslundii; dilution 1:100 for S. sanguis; dilution 1:20 for F. nucleatum; and dilution 1:20 for V. parvula). For the two obligate anaerobic strains, F. nucleatum and V. parvula, the BHI medium is supplemented with Oxyrase (100 .mu.l/5 ml). Oxyrase for Broth is a sterile enzyme additive which is used to produce anaerobic conditions in a wide variety of bacteriological broth medium. The cells in the BHI broth are added to 96 well plates at a volume of 180 .mu.l/well. The compounds to be tested are added to the wells (20 .mu.l/well) to give final assay concentrations over the desired range. The plates are incubated at 37.degree. C. for specific period of time, determined separately for each bacterial culture. After the incubation period the optical density is measured using a Bio-Tek EL 340 Microplate Biokinetics.RTM. reader. For studies carried out with Alamar Blue.RTM. to monitor the growth of the cultures, fluorescence is measured using a Tecan Spectrafluor.RTM. fluorescence plate reader.

US 20030077232A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2003/0077232 A1**

Cromwell et al. (43) **Pub. Date: Apr. 24, 2003**

(54) **COMPOSITION**

(75) Inventors: **Victoria Cromwell**, Merseyside (GB); **Peter Freunscht**, Merseyside (GB); **Peter John Hall**, Merseyside (GB); **David Thomas Littlewood**, Merseyside (GB)

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(73) Assignee: **Unilever Home & Personal Care USA, Division of Conopco, Inc.**

(21) Appl. No.: **10/225,857**

(22) Filed: **Aug. 22, 2002**

(30) **Foreign Application Priority Data**

Aug. 24, 2001 (EP) 01307269.9
Dec. 11, 2001 (EP) 01310338.7

Publication Classification

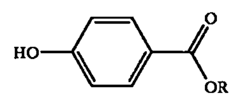
(51) **Int. Cl.**[7] **A61K 9/68**; A61K 7/16

(52) **U.S. Cl.** **424/48**; 424/49

(57) **ABSTRACT**

Oral composition comprising an alkyl hydroxybenzoate represented by formula I

Formula I:



wherein R represents a straight chain alkyl group comprising at least eight carbon atoms.

[0655] Freeze-dried vials of Clostridium sordellii purchased from ATCC were placed in a bovine bone that contained four holes with a diameter slightly greater than the circumference of the vials that extended to the midpoint of the bone. The bone containing the vials was then irradiated at 1.5 kGy/hr with 0, 25 or 50 kGy of gamma radiation ~~at either 4.degree~~. C. or on dry ice. The contents of the vials were then resuspended in 10 mL of Reinforced Clostridial Medium supplemented with Oxyrase to provide an anaerobic environment. ~~Serial ten-fold dilutions were made to a dilution of 10.sup.-9.~~ Fifty microliters of each dilution was then spread on a plate containing Reinforced Clostridial Medium plus 1.5% agar. A BBL GasPak Anaerobic System was used to provide an anaerobic environment for growth of the plated bacteria. The broth cultures and the plates were incubated at 37.degree. C. for 48 hours. Following incubation turbidity was visualized and absorbance readings were taken at 620 nm in the broth cultures and colonies were counted on the plates. Similar cultures of Staph. epidermidis and E. coli were also set up and irradiated. These cultures were prepared using media and conditions conventional for the organisms.

7

DOCUMENT-IDENTIFIER: US 20040033160 A1
TITLE: Methods for sterilizing biological materials by irradiation over a temperature gradient

L5: Entry 1 of 1 File: PGPB Mar 11, 2004

DOCUMENT-IDENTIFIER: US 20040048778 A1
TITLE: Selective growth media

Detail Description Paragraph:
[0085] Salmonella typhimurium (OCC 1792) was heat stressed at 51.4.degree. C. for 25 minutes according to the protocol of Stephens et al. (1997 J. Appl. Micro. 8, 445-455). Cells were diluted in 7 different resuscitation media: yeast extract broth ~~with a~~ high reactive oxygen species content that is inhibitory to stressed cells, SPRINT enrichment broth supplemented with Oxyrase that is an optimised resuscitation medium, BPW as the control that has typical resuscitation properties, BPW supplemented with NaCl that is inhibitory to stressed cells, and BPW supplemented with 3 different levels of AEP. Resuscitation was quantified using a microtitre MPN method.

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L5: Entry 1 of 1 File: PGPB Mar 11, 2004

PGPUB-DOCUMENT-NUMBER: 20040048778
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040048778 A1

TITLE: Selective growth media

PUBLICATION-DATE: March 11, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Druggan, Patrick	Basingstoke		GB

APPL-NO: 10/380330 [PALM]
DATE FILED: September 12, 2003

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
GB	0022556.5	2000GB-0022556.5	September 14, 2000

PCT-DATA:

DATE-FILED	APPL-NO	PUB-NO	PUB-DATE	371-DATE	102(E)-DATE
Sep 14, 2001	PCT/GB01/04124				

INT-CL-PUBLISHED: [07] A61 K 38/17

US-CL-PUBLISHED: 514/002
US-CL-CURRENT: 514/2

REPRESENTATIVE-FIGURES: NONE

ABSTRACT:

Disclosed is a method of selectively inhibiting for growth of non-target cells in a mixed population of target and non-target cells, the method comprising the steps of: (a) contacting the mixed population with a selective agent which comprises a carrier moiety linked by a scissile linkage to a toxic moiety; wherein the selective agent is able to enter non-target cells in which the scissile linkage is cleaved, releasing the toxic moiety to exert a toxic effect on the non-target cells causing inhibition of the growth of the non-target cells, whereas the selective agent is unable to enter target cells and/or the scissile linkage is not cleaved in target cells and/or toxic moiety, if released from the selective agent, does not exert a toxic effect on the target cell; and (b) culturing the cells in conditions which allow for growth of non-inhibited cells.

US 20040048778A1

(19) United States
(12) Patent Application Publication
(10) Pub. No.: US 2004/0048778 A1
(43) Pub. Date: Mar. 11, 2004
Druggan

(54) SELECTIVE GROWTH MEDIA

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WASHINGTON, DC 20004 (US)

(21) Appl. No.: **10/380,330**

(22) PCT Filed: **Sep. 14, 2001**

(86) PCT No.: **PCT/GB01/04124**

(30) **Foreign Application Priority Data**

Sep. 14, 2000 (GB) 0022556.5

Publication Classification

(51) **Int. Cl.^{7}** **A61K 38/17**

(52) **U.S. Cl.** **514/2**

(57) **ABSTRACT**

Disclosed is a method of selectively inhibiting for growth of non-target cells in a mixed population of target and non-target cells, the method comprising the steps of: (a) contacting the mixed population with a selective agent which comprises a carrier moiety linked by a scissile linkage to a toxic moiety; wherein the selective agent is able to enter non-target cells in which the scissile linkage is cleaved, releasing the toxic moiety to exert a toxic effect on the non-target cells causing inhibition of the growth of the non-target cells, whereas the selective agent is unable to enter target cells and/or the scissile linkage is not cleaved in target cells and/or toxic moiety, if released from the selective agent, does not exert a toxic effect on the target cell; and (b) culturing the cells in conditions which allow for growth of non-inhibited cells.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/16677

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12M 1/00, 1/24, 1/34, 3/00; C12Q 1/00; G01N 33/53, 33/554
US CL : 435/4, 7.2, 7.32, 283.1, 287.1, 287.3, 288.1, 288.3, 289.1, 303.1, 303.2, 304.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/4, 7.2, 7.32, 283.1, 287.1, 287.3, 288.1, 288.3, 289.1, 303.1, 303.2, 304.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	POST et al. Simple Medium for the Selective Isolation of Bacteroides and Related Organisms, and Their Occurenece in Sewage. Applied Microbiology. March 1967, Vol. 15, No. 2, pages 213-218, especially page 213.	1-35

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search
15 January 2003 (15.01.2003)

Date of mailing of the international search report
14 MAR 2003

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Form PCT/ISA/210 (second sheet) (July 1998)

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L6: Entry 17 of 17 File: USPT Mar 28, 1995

US-PAT-NO: 5401639
DOCUMENT-IDENTIFIER: US 5401639 A

TITLE: Stabilized bilirubin calibrator solution and method therefor

DATE-ISSUED: March 28, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Saldivar, Jr.; Louis	Kenosha	WI		
England; Barbara J.	Milwaukee	WI		

US-CL-CURRENT: 435/14; 435/801, 436/97

CLAIMS:

We claim:

1. A stabilized aqueous bilirubin calibrator solution comprising:

between about 1.0 and 30 mg/dl of bilirubin;

between about 0.9 and 1.1 weight percent ethanol;

between about 5 and 6 weight percent polyoxyethylene 23 lauryl ether;

between about 0.0016 and 0.024 weight percent butylated hydroxy toluene;

bistrispropane at about 0.1M;

between about 0.1 and 0.53 weight percent gluconic acid generated in situ from glucose;

between about 4 and 6 weight percent albumin;

d-lactate hydrogen donor at between about 3 and 20 mM; and

between about 0.3 and 0.6 units/ml sterile membrane fragments derived from bacteria having membranes containing an oxygen transfer system which reduces oxygen to water;

wherein said stabilized aqueous bilirubin calibrator solution has a pH from about 7.7 to about 7.85 and has a storage stability of at least six months at from about 2.degree. to about 8.degree. C. and at least four days at 45.degree. C., said calibrator solution further having an open vial stability of at least three weeks at from about 2.degree. to about 8.degree. C.

biochemical pathway act in a coordinated fashion to produce a specific product or products or to produce some other particular biochemical action. Such a biochemical pathway requires the expression product of a gene if the absence of that expression product either directly or indirectly prevents the completion of one or more steps in that pathway, thereby preventing or significantly reducing the production of one or more normal products or effects of that pathway. Thus, an agent specifically inhibits such a biochemical pathway requiring the expression product of a particular gene if the presence of the agent stops or substantially reduces the completion of the series of steps in that pathway. Such an agent, may, but does not necessarily, act directly on the expression product of that particular gene.

Brief Summary Text (56):
In another related aspect, the invention provides a method of inhibiting the growth of a pathogenic bacterium by contacting the bacterium with an antibacterial agent which specifically inhibits a biochemical pathway requiring the expression product of a gene selected from the group of genes corresponding to SEQ ID NO. 1-3 or a homologous gene. Inhibition of that pathway inhibits growth of the bacterium. In particular embodiments, the antibacterial agent inhibits the expression product of one of the identified genes.

Brief Summary Text (57):
The term "inhibiting the growth" indicates that the rate of increase in the numbers of a population of a particular bacterium is reduced. Thus, the term includes situations in which the bacterial population increases but at a reduced rate, as well as situations where the growth of the population is stopped, as well as situations where the numbers of the bacteria in the population are reduced or the population even eliminated.

Drawing Description Text (7):
FIG. 6 shows the percent inhibition of growth by a natural product extracts screening hit (HEPA screen) at a range of concentrations against 3 S. aureus strains, wild-type strain 8325-4, NT372 ts mutant, and the complemented ts mutant, SAM533.

Detailed Description Text (31):
Mutant NT372 was initially identified by its inability to survive a temperature shift to 43.degree. C. on TSA plates for 2 hours. The ts phenotype is partially rescued at 43.degree. C. by plating either on TSA plates supplemented with 1M NaCl or Oxyrase; subsequent reselection of the surviving colonies on TSA alone at 43.degree. C. reconfirms the ts phenotype. The phenotype does not seem to be linked to carbon source.

Detailed Description Text (55):
Specific examples of methods for identifying homologous genes are described in Van Dijl et al., U.S. Pat. No. 5,246,838, issued Sep. 21, 1993. In addition to the direct hybridization methods for identifying and isolating homologous genes mentioned above, Van Dijl et al. describe the isolation of homologous genes by isolating clones of a host bacterial strain which contain random DNA fragments from a donor microorganism. In those clones a specific host gene has been inactivated (such as by linkage with a regulatable promoter), and inserted homologous genes are identified by the complementation of the inactivated gene function. Homologous genes identified in this way can then be sequenced.

Detailed Description Text (60):
While the identification of a particular bacterial gene as an essential gene for growth in a rich medium characterizes that gene as an antibacterial target, it is useful to characterize the gene further in order to prioritize the targets. This process is useful since it allows further work to be focused on those targets with the greatest therapeutic potential. Thus, target genes are prioritized according to which are more likely to allow identification of antibacterial agents which are: 1.

L6: Entry 11 of 17 File: USPT Feb 4, 2003

DOCUMENT-IDENTIFIER: US 6514746 B1
TITLE: Staphylococcus aureus histidine protein kinase essential genes

Brief Summary Text (6):
The second approach involves the identification of new targets, and the subsequent screening of compounds to find antibacterial agents affecting those targets. Such screening can involve any of a variety of methods, including screening for inhibitors of the expression of a gene, or of the product of a gene, or of a pathway requiring that product. However, generally the actual target is a protein, the inhibition of which prevents the growth or pathogenesis of the bacterium. Such protein targets can be identified by identifying genes encoding proteins essential for bacterial growth.

Brief Summary Text (14):
The term "active against" in the context of compounds, agents, or compositions having antibacterial activity indicates that the compound exerts an effect on a particular bacterial target or targets which is deleterious to the in vitro and/or in vivo growth of a bacterium having that target or targets. In particular, a compound active against a bacterial gene exerts an action on a target which affects an expression product of that gene. This does not necessarily mean that the compound acts directly on the expression product of the gene, but instead indicates that the compound affects the expression product in a deleterious manner. Thus, the direct target of the compound may be, for example, at an upstream component which reduces transcription from the gene, resulting in a lower level of expression. Likewise, the compound may affect the level of translation of a polypeptide expression product, or may act on a downstream component of a biochemical pathway in which the expression product of the gene has a major biological role. Consequently, such a compound can be said to be active against the bacterial gene, against the bacterial gene product, or against the related component either upstream or downstream of that gene or expression product. While the term "active against" encompasses a range of potential activities, it also implies some degree of specificity of target. Therefore, for example, a general protease is not "active against" a particular bacterial gene which produces a polypeptide product. In contrast, a compound which specifically inhibits a particular enzyme is active against that enzyme and against the bacterial gene which codes for that enzyme.

Brief Summary Text (54):
In a related aspect, the invention provides a method for treating a bacterial infection in a mammal by administering an amount of an antibacterial agent effective to reduce the infection. The antibacterial agent specifically inhibits a biochemical pathway requiring the expression product of a gene corresponding to one of the genes identified in the first aspect above. Inhibition of that pathway inhibits the growth of the bacteria in vivo. In particular embodiments, the antibacterial agent inhibits the expression product of one of the identified genes.

Brief Summary Text (55):
In this context, the term "biochemical pathway" refers to a connected series of biochemical reactions normally occurring in a cell, or more broadly a cellular event such as cellular division or DNA replication. Typically, the steps in such a

Highly inhibitory to the target in relevant pathogenic species; 2. Cause rapid loss of bacterial viability; 3. Not have frequently arising resistance mechanisms; 4. Have high selectivity for the bacterial target and little, or preferably no, effect on the related mammalian targets; 5. Have low non-specific toxicity to mammals; and 6. Have appropriate pharmacodynamic and physical properties for use as a drug. Consequently, target genes are prioritized using a variety of methods, such as those described below.

Detailed Description Text (62):
Essential genes can be characterized as either bactericidal or bacteriostatic. Earlier work with Salmonella mutants established that the bactericidal/bacteriostatic distinction was a characteristic of inhibition of the specific gene, rather than of a mutant allele, and could be characterized in vitro. (Schmid et al., 1989, Genetics 123:625-633.) Therefore, preferred targets (high priority) are those which are highly bactericidal when inhibited, causing cell death. A subset of the bactericidal essential genes can be identified as strongly bactericidal, resulting in rapid cell death when inhibited.

Detailed Description Text (63):
In S. typhimurium, inhibition of strongly bactericidal genes was shown to result in one of the following effects: 1. Cell lysis (such genes generally involved in cell wall biosynthesis); 2. Inhibition of protein synthesis; 3. DNA degradation; or 4. Entry into non-recoverable state involving cell cycle related genes.

Detailed Description Text (86):
In addition to identifying new targets for drug discovery, the growth conditional mutants are useful for screening for inhibitors of the identified targets, even before the novel genes or biochemical targets are fully characterized. The methodology can be whole-cell based, is more sensitive than traditional screens searching for strict growth inhibitors, can be tuned to provide high target specificity, and can be structured so that more biological information on test compounds is available early for evaluation and relative prioritization of hits.

Detailed Description Text (87):
Certain of the screening methods are based on the hypersensitivity of growth conditional mutants. For example, conditionally lethal ts mutants having temperature sensitive essential gene functions are partially defective at a semi-permissive temperature. As the growth temperature is raised, the mutated gene causes a progressively crippled cellular function. It is the inherent phenotypic properties of such ts mutants that are exploited for inhibitor screening.

Detailed Description Text (88):
Each temperature sensitive mutant has secondary phenotypes arising from the genetic and physiological effects of the defective cellular component. The genetic defect causes a partially functional protein that is more readily inhibited by drugs than the wild type protein. This specific hypersensitivity can be exploited for screening purposes by establishing "genetic potentiation" screens. In such screens, compounds are sought that cause growth inhibition of a mutant strain, but not of wild type, or greater inhibition of the growth of a mutant strain than of a wild type strain. Such compounds are often (or always) inhibitors of the wild type strain at higher concentrations.

Detailed Description Text (89):
Also, the primary genetic defect can cause far-reaching physiological changes in the mutant cells, even in semi-permissive conditions. Necessity for full function of biochemically related proteins upstream and downstream of the primary target may arise. Such effects cause hypersensitivity to agents that inhibit these related proteins, in addition to agents that inhibit the genetically defective cellular component. The effects of the physiological imbalance will occur through metabolic interrelationships that can be referred to as the "metabolic web". Thus, in some

cases, the initial genetic potentiation screen has the ability to identify inhibitors of either the primary target, or biochemically related essential gene targets.

Detailed Description Text (94):
In addition to single strain screening, growth conditional mutants such as ts mutants, can be used in sets to provide compoud specific susceptibility profiles. As a screening tool, the mutant inhibition profile characterizes the effects of test compounds on specific bacterial pathways. Because the mutants are more sensitive than wild type strains, compounds with weak inhibition activity can be identified.

Detailed Description Text (97):
Certain testing parameters for the genetic potentiation screening methods can significantly affect the identification of growth inhibitors, and thus can be manipulated to optimize screening efficiency and/or reliabilty. Notable among these factors are variable thermosensitivity of different ts mutants, increasing hypersensititivy with increasing temperature, and "apparent" increase in hypersensitivity with increasing compound concentration.

Detailed Description Text (101):
The ts mutants are more sensitive to potential inhibitors at elevated temperature. This temperature effect can be used to control hit rates in the screening. Higher screening temperature can be used to produce more hits for mutants that have low hit rates. Similarly, if a mutant shows a very high hit rate, the number of hits can be reduced by using lower screening temperatures to facilitate hit prioritization.

Detailed Description Text (103):
The concentration of compounds used in the screening is an important parameter in determining the hit rates and the amount of follow-up studies. The concentration of 10 .mu.g/ml has been used in piloting screening studies. Screening at concentrations <2 .mu.g/ml may miss at least half of the hits that would be identified at 10 .mu.g/ml. On the other hand, screening at concentrations higher than 10 .mu.g/ml may result in a large number of low quality hits and create too much work in hit confirmation and follow-up studies. At 10 .mu.g/ml, a hit may appear as a growth inhibitor for both the mutant and wild type strains. This should not be a major problem since lower concentrations of the compound can be tested in the follow-up studies to differentiate its effect on the mutant and the wild type.

Detailed Description Text (105):
The methods of this invention are suitable and useful for screening a variety of sources for possible activity as inhibitors. For example, compound libraries can be screened, such as natural product libraries, combinatorial libraries, or other small molecule libraries. In addition, compounds from commercial sources can be tested, this testing is particularly appropriate for commercially available analogs of identified inhibitors of particular bacterial genes.

Detailed Description Text (106):
Compounds with identified structures from commercial sources can be efficiently screened for activity against a particular target by first restricting the compounds to be screened to those with preferred structural characteristics. As an example, compounds with structural characteristics causing high gross toxicity can be excluded. Similarly, once a number of inhibitors of a specific target have been found, a sub-library may be generated consisting of compounds which have structural features in common with the identified inhibitors. In order to expedite this effort, the ISIS computer program (MDL Information Systems, Inc.) is suitable to perform a 2D-substructure search of the Available Chemicals Directory database (MDL Information Systems, Inc.). This database contains structural and ordering information on approximately 175,000 commercially available chemical compounds.

Other publicly accessible chemical databases may similarly be used.

Detailed Description Text (108):
Gross acute toxicity of an identified inhibitor of a specific gene target may be assessed in a mouse model. The inhibitor is administered at a range of doses, including high doses, (typically 0-100 mg/kg, but preferably to at least 100 times the expected therapeutic dose) subcutaneously or orally, as appropriate, to healthy mice. The mice are observed for 3-10 days. In the same way, a combination of such an inhibitor with any additional therapeutic components is tested for possible acute toxicity.

Detailed Description Text (110):
The particular compound that is an antibacterial agent can be administered to a patient either by itself, or in combination with another antibacterial agent, or in pharmaceutical compositions where it is mixed with suitable carriers or excipient (s). A combination of an inhibitor of a particular gene with another antibacterial agent can be of at least two different types. In one, a quantity of an inhibitor is combined with a quantity of the other antibacterial agent in a mixture, e.g., in a solution or powder mixture. In such mixtures, the relative quantities of the inhibitor and the other antibacterial agent may be varied as appropriate for the specific combination and expected treatment. In a second type of combination an inhibitor and another antibacterial agent can be covalently linked in such manner that the linked molecule can be cleaved within the cell. However, the term "in combination" can also refer to other possibilities, including serial administration of an inhibitor and another antibacterial agent. In addition, an inhibitor and/or another antibacterial agent may be administered in pro-drug forms, i.e. the compound is administered in a form which is modified within the cell to produce the functional form. In treating a patient exhibiting a disorder of interest, a therapeutically effective amount of an agent or agents such as these is administered. A therapeutically effective dose refers to that amount of the compound(s) that results in amelioration of symptoms or a prolongation of survival in a patient, and may include elimination of a microbial infection.

Detailed Description Text (111):
Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD.sub.50 (the dose lethal to 50% of the population) and the ED.sub.50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD.sub.50 /ED.sub.50. Compounds which exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED.sub.50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. It is preferable that the therapeutic serum concentration of an EspA/EspB inhibitor should be in the range of 0.1-100 .mu.g/ml.

Detailed Description Text (126):
The essential sensor pair espAB is used as the target of the HEPA screen (hypersensitivity in the espAB pathway), which is designated to detect inhibitors of EspA and/or EspB function. The expected cellular location and general function of EspA and B is shown in FIG. 5, indicating that the EspB product is believed to locate to the cell membrane. The HEPA screen is a genetic potentiation screen and is summarized in the following.

Detailed Description Text (127):
A. HEPA screen: Initial Identification of Inhibitors

Detailed Description Text (129):

Hit-compounds were defined by the relative percentage of growth inhibition of NT372 and isogenic wild type cells when both cell types are exposed to a given test compound. Percent inhibition for each strain is calculated as: [1-(OD600 cpd well--OD600 blank well)/(OD600 no cpd well--OD600 blank well].times. 100. In screening synthetic compounds, hits are defined as those compounds that inhibit the growth of NT372 .gtoreq.70% and WT cells <90%. In screening natural products, hits are defined as class AA (%Inh.sub.NT372.gtoreq.70% and %Inh.sub.NT372 --% Inh.sub.wt.gtoreq.90)or class A (%Inh.sub.NT372.gtoreq.70% and %Inh.sub.NT372 --% Inh.sub.wt.gtoreq.70).

Detailed Description Text (132):
Hit evaluation can be extended through the use of biochemical assays to determine if hit compounds specifically affect the functioning of purified EspA and EspB components. The companion in vitro biochemical screen is set up using purified EspA and EspB proteins, which can be achieved expressing the espAB gene sequences. Leads identified from the specificity-titration assay will be examined for acceptable chemical structure, then utilized for analysis in the EspAB biochemical assay. This strategy for Stage I combines the powerful screening ability of a genetic-based search for EspAB inhibitors with a direct demonstration of EspAB targeting using the purified proteins.

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Other Reference Publication (40):
Sanger et al., "DNA sequencing with chain-terminating inhibitors," Proc. Natl. Acad. Sci. USA 74:5463-5467 (1977).

Previous Doc Next Doc Go to Doc#

US 20030138874A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2003/0138874 A1

Taintor (43) Pub. Date: Jul. 24, 2003

(54) METHOD AND KIT FOR RAPID CONCURRENT IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY TESTING OF MICROORGANISMS FROM BROTH CULTURE

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(21) Appl. No.: 10/036,042

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Publication Classification

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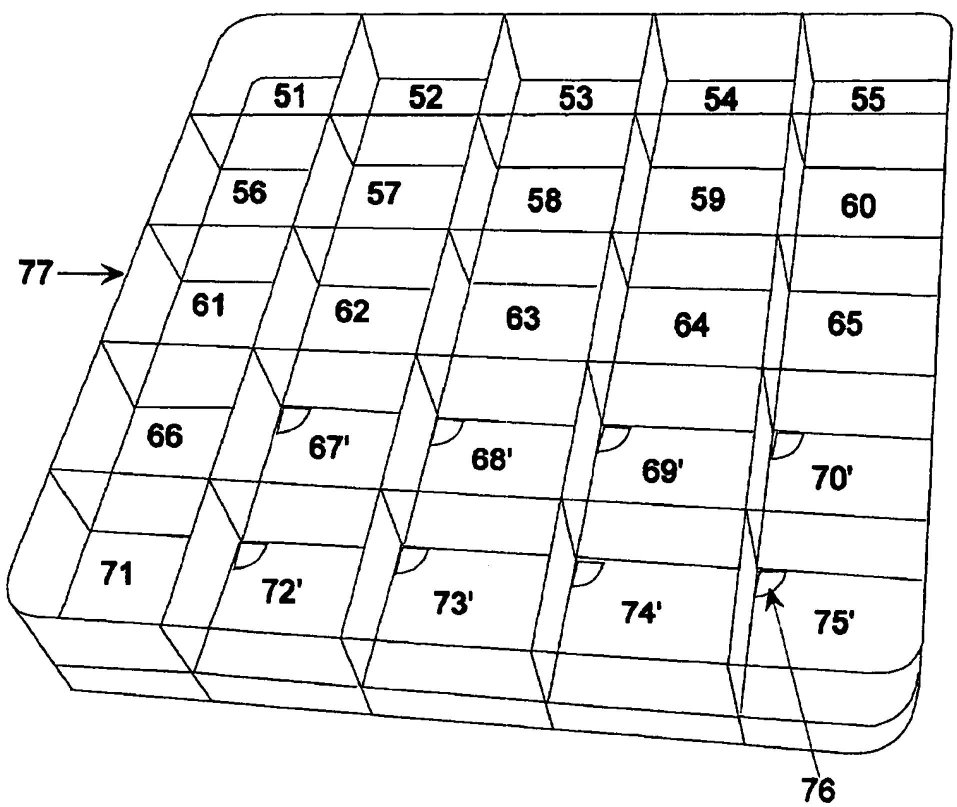
(52) U.S. Cl. 435/34

(57) ABSTRACT

A Method and Kit for performing concurrent identification testing and antimicrobial susceptibility testing from broth culture (90) are described. Broth (82) incubation is generally 4 to 6 hrs providing adequate numbers of microorganisms for inoculating a multi-chambered plate (80) comprising enriched, differential, selective, differential-selective, single-purpose and susceptibility media. Several dilutions are prepared from the cultured broth, for inoculation of the kit plate (80). The more dilute concentration (140) produces individual colonies of microorganisms, for identification testing. This isolation makes an initial isolation step unnecessary. The heavier concentration dilution (96) provides inoculation for antimicrobial susceptibility tests and other identification tests. In addition, antimicrobial susceptibilities are shown valid even when several different microorganisms coexist in the same test chamber. The method is fast for bacteria, providing identification and susceptibility data in 24 hrs. The kit is complete, except for an incubator and microscope. The method is simple to perform and can be utilized almost anywhere.



US006429008B1

(12) United States Patent
Copeland et al.

(10) Patent No.: **US 6,429,008 B1**
(45) Date of Patent: ***Aug. 6, 2002**

(54) **APPARATUS AND METHOD FOR GROWING ANAEROBIC MICROORGANISMS**

(75) Inventors: **James C. Copeland**, Ashland, OH (US); **Howard I. Adler**, deceased, late of Oak Ridge, by Martha Vogeler Adler, executrix; **Gerald E. Spady**, Oak Ridge, both of TN (US)

(73) Assignee: **Oxyrase, Inc.**, Mansfield, OH (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **09/812,204**

(22) Filed: **Mar. 19, 2001**

Related U.S. Application Data

(63) Continuation of application No. 09/321,812, filed on May 28, 1999, now Pat. No. 6,204,051, which is a continuation-in-part of application No. 08/963,664, filed on Nov. 3, 1997, now Pat. No. 5,955,344, which is a continuation of application No. 08/237,773, filed on May 4, 1994, now Pat. No. 5,830,746.

(51) **Int. Cl.**^{7} **C12M 1/00**

(52) **U.S. Cl.** **435/303.2**; 435/305.4; 435/307.1; 435/801; 422/102

(58) **Field of Search** 435/288.3, 303.2, 435/305.4, 307.1, 801; 422/102

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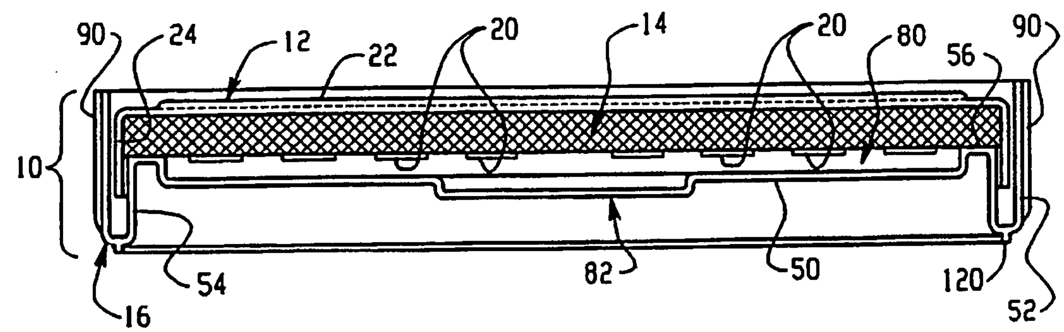
Primary Examiner—David A. Redding
(74) *Attorney, Agent, or Firm*—Fay, Sharpe, Fagan, Minnich & McKee LLP

(57) **ABSTRACT**

An apparatus for growing anaerobic microorganisms is provided having a dish top that contains a sealing ring upon which the media surface in the dish bottom rests when the apparatus is inverted. The contact between the sealing ring and the media surface forms a seal that traps the gas in the headspace between the media surface and the inside of the dish top. A oxygen reducing agent can also be incorporated into the media together, in some instances, with a substrate which react with oxygen in the media and with oxygen in the headspace thereby creating an environment suitable for growing anaerobic, microaerophilic and facultative anaerobic microorganisms.

22 Claims, 8 Drawing Sheets



US005482860A

United States Patent [19]

Copeland et al.

[11] **Patent Number: 5,482,860**

[45] **Date of Patent: Jan. 9, 1996**

[54] **APPARATUS FOR CONTINUOUSLY REMOVING OXYGEN FROM FLUID STREAMS**

[75] Inventors: **James C. Copeland**, Ashland, Ohio; **Howard I. Adler**, Oak Ridge, Tenn.

[73] Assignee: **Oxyrase, Inc.**, Ashland, Ohio

[21] Appl. No.: **49,995**

[22] Filed: **Apr. 20, 1993**

Related U.S. Application Data

[62] Division of Ser. No. 319,748, Mar. 7, 1989, Pat. No. 5,240,843.

[51] **Int. Cl.**[6] **C12M 1/40**

[52] **U.S. Cl.** **435/293.1**; 435/813; 435/297.1

[58] **Field of Search** 435/175, 182, 435/262, 288, 299, 300, 310, 311, 313, 801, 813, 820; 210/632, 150, 151

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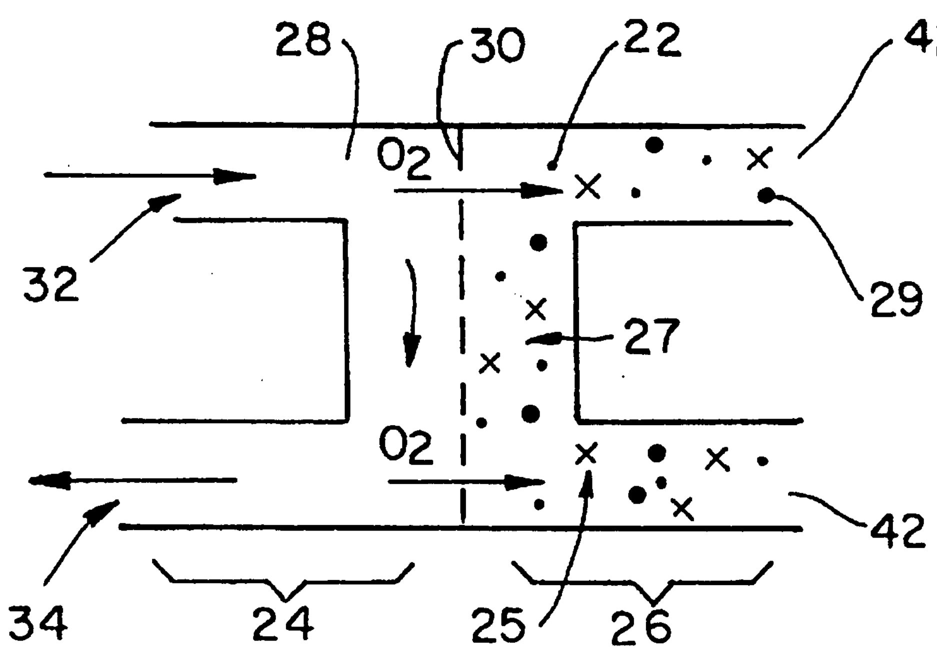
Primary Examiner—William H. Beisner
Attorney, Agent, or Firm—Fay, Sharpe, Beall, Fagan, Minnich & McKee

[57] **ABSTRACT**

The present invention is directed to an apparatus for removing oxygen from a fluid stream. The apparatus is comprised of a flow through reactor chamber containing a sufficient amount of oxygen scavenging cell membrane fragments to catalyze the transformation of oxygen present in a fluid stream to water. The fragments are contained in the apparatus in a manner which allows free contact between the fragments and the fluid stream flowing therethrough or for the diffusion of the oxygen from the fluid stream across a synthetic membrane to a compartment containing the fragments where the oxygen is transformed to water. An inlet for introducing a fluid stream containing oxygen into said flow-through reaction chamber and an outlet for removing the deoxygenated fluid stream are also provided.

12 Claims, 3 Drawing Sheets



US006204051B1

(12) **United States Patent**
Copeland et al.

(10) **Patent No.:** **US 6,204,051 B1**
(45) **Date of Patent:** ***Mar. 20, 2001**

(54) **APPARATUS AND METHOD FOR GROWING ANAEROBIC MICROORGANISMS**

(75) Inventors: **James C. Copeland**, Ashland, OH (US); **Howard I. Adler**, deceased, late of Oak Ridge, by Martha Vogeler Adler, legal representative; **Gerald E. Spady**, Oak Ridge, both of TN (US)

(73) Assignee: **Oxyrase, Inc.**, Mansfield, OH (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **09/321,812**

(22) Filed: **May 28, 1999**

Related U.S. Application Data

(63) Continuation-in-part of application No. 08/963,664, filed on Nov. 3, 1997, now Pat. No. 5,955,344, which is a continuation of application No. 08/237,773, filed on May 4, 1994, now Pat. No. 5,830,746.

(51) **Int. Cl.**[7] **C12M 1/22**

(52) **U.S. Cl.** **435/305.4**; 435/288.3; 435/303.2; 435/801

(58) **Field of Search** 435/288.3, 305.1, 435/305.4, 307.1, 303.2, 801

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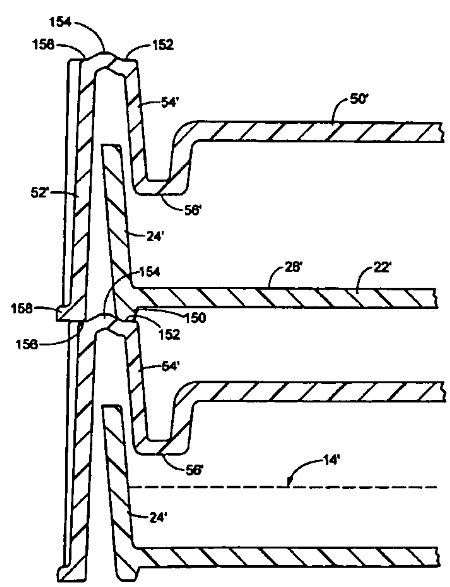
Primary Examiner—David A. Redding
(74) *Attorney, Agent, or Firm*—Fay, Sharpe, Fagan, Minnich & McKee, LLP

(57) **ABSTRACT**

An apparatus for growing anaerobic microorganisms is provided having a dish top that contains a sealing ring upon which the media surface in the dish bottom rests when the apparatus is inverted. The contact between the sealing ring and the media surface forms a seal that traps the gas in the headspace between the media surface and the inside of the dish top. A oxygen reducing agent can also be incorporated into the media together, in some instances, with a substrate which react with oxygen in the media and with oxygen in the headspace thereby creating an environment suitable for growing anaerobic, microaerophilic and facultative anaerobic microorganisms.

20 Claims, 8 Drawing Sheets



United States Patent [19]
Adler
[11] Patent Number: 4,476,224
[45] Date of Patent: Oct. 9, 1984

[54] MATERIAL AND METHOD FOR PROMOTING THE GROWTH OF ANAEROBIC BACTERIA

[76] Inventor: Howard I. Adler, 128 Indian La., Oak Ridge, Tenn. 37830

[21] Appl. No.: 376,640
[22] Filed: May 10, 1982
[51] Int. Cl.³ C12N 1/20
[52] U.S. Cl. 435/253; 435/801; 435/820
[58] Field of Search 435/253, 801, 189, 244, 435/253, 260, 262, 317, 820, 190, 191, 192; 426/8

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Primary Examiner—Robert J. Warden
Assistant Examiner—Randall E. Deck
Attorney, Agent, or Firm—Luedeka & Neely

[57] ABSTRACT

A material and method for promoting the growth of anaerobic bacteria which includes a nutrient media containing a hydrogen donor and sterile membrane fragments of bacteria having an electron transfer system which reduces oxygen to water. Dissolved oxygen in the medium is removed by adding the sterile membrane fragments to the nutrient medium and holding the medium at a temperature of about 10° to about 60° C. until the dissolved oxygen is removed.

10 Claims, No Drawings